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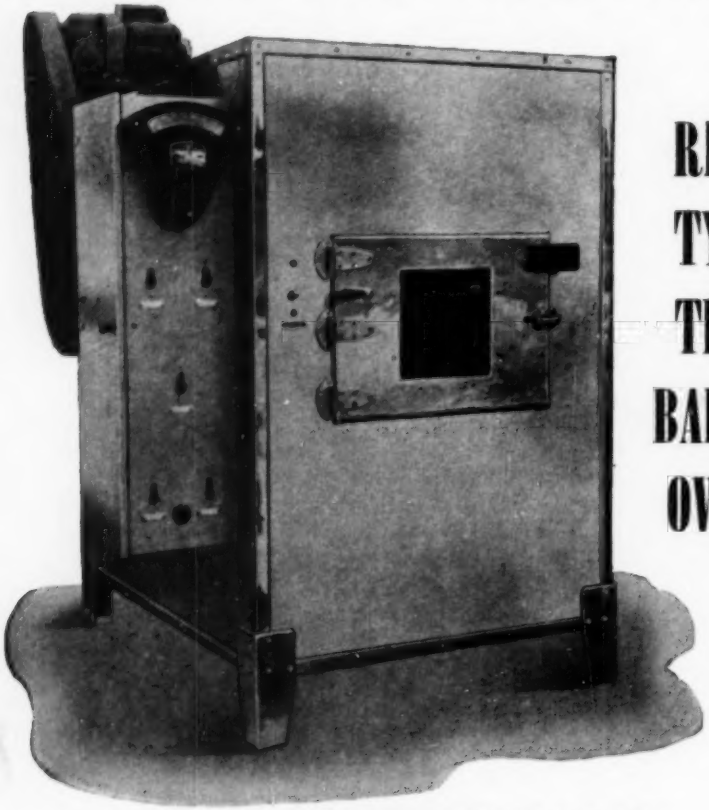

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Schaumburg, Sandstedt, and Fleming:

Page 45, Fig. 1, line 2: *For* × 30 *read* × 27.

Page 46, Fig. 2, line 2: *For* × 400 *read* × 350.

Page 47, Fig. 3, line 2: *For* × 400 *read* × 360.

Page 47, paragraph 2, line 6: *For* × 200 *read* × 170.

Page 47, Fig. 4, line 2: *For* × 200 *read* × 170.

Page 48, line 3: *For* × 400 *read* × 380.

Page 48, Fig. 5, line 2: *For* × 400 *read* × 380.

Gordon, Jenness, and Geddes:

Page 5: To replace Table I, please cut out the following corrected table and paste it over the original one.

TABLE I

EFFECT OF CASEINATE SOLS AND OF SALT SOLUTIONS ON LOAF VOLUME

Dough Supplement	Calcium Hydroxide per 3 g. Casein	pH of Supplement	Mean Loaf Volume[1]
	mg.		*cc.*
None—control			788
Sodium caseinate[2]	0	6.6	618
Calcium caseinate[2]	42	6.5	628
Calcium caseinate[2]	84	6.5	625
Calcium caseinate[2]	182	6.5	613
Acid casein[2] in 80 ml. salt solution		6.0	623
40 ml. salt solution[3]		6.0	772
80 ml. salt solution[3]		6.0	743

[1] Each value represents the average of duplicates. The standard error of a single determination in this series was 11.1 cc.

[2] Three g. of casein per 200 g. flour.

[3] Solution consisted of 0.460 g. $CaCl_2 \cdot 2H_2O$, 0.150 g. Na_2HPO_4, and 0.124 g. $NaH_2PO_4 \cdot H_2O$ per 100 ml. Thus it contained 125 mg. each of calcium and phosphate (as PO_4) per 100 ml. A fine flocculent precipitate formed in this mixture. It was mixed thoroughly before use.

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Enriched BREAD, or other baked products	1.1	1.8	0.7	1.6	10.0	15.0	8.0	12.5
Enriched FLOUR[1]	2.0	2.5	1.2	1.5	16.0	20.0	13.0	16.5
Enriched FARINA[2]	1.66	—	1.2	—	6.0	—	6.0	—
Enriched MACARONI products[3]	4.0	5.0	1.7	2.2	27.0	34.0	13.0	16.5
Enriched NOODLE products[3]	4.0	5.0	1.7	2.2	27.0	34.0	13.0	16.5
Enriched CORN MEALS	2.0	3.0	1.2	1.8	16.0	24.0	13.0	26.0
Enriched CORN GRITS[4]	2.0	3.0	1.2	1.8	16.0	24.0	13.0	26.0
Enriched MILLED WHITE RICE[5]	2.0	—	*		16.0	—	13.0	—

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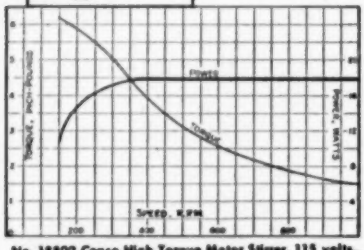

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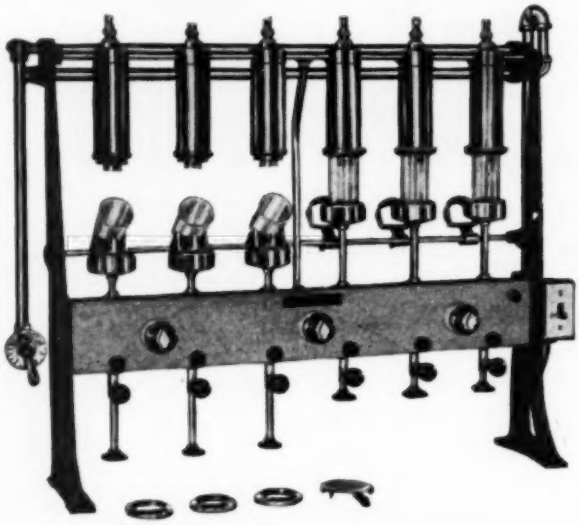

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CEREAL CHEMISTRY

VOL. XXXI JANUARY, 1954 NO. 1

THE BAKING BEHAVIOR OF CASEIN AND WHEY PREPARED FROM SKIMMILK BY VARIOUS PROCEDURES[1]

A. L. GORDON,[2] ROBERT JENNESS,[3] AND W. F. GEDDES[3]

ABSTRACT

Casein was separated from raw skimmilk by acid coagulation, rennet coagulation, and supercentrifugation. Baking tests, in which the caseins and corresponding wheys were incorporated into bread doughs in quantities equivalent to those which would be added if 6% of nonfat milk solids were included in the formula, showed that all fractions depressed the loaf volume of bread made without shortening. The casein preparations were more deleterious to baking quality than the wheys. The method of fractionation had little influence, although acid-precipitated casein gave loaf volumes slightly superior to those given by casein prepared by rennet coagulation or supercentrifugation. Baking tests made with sodium caseinate and three calcium caseinate sols containing different levels of calcium gave similar results; only a slight depression in loaf volume was produced by a calcium phosphate suspension.

These results confirm the observations of certain Dutch workers (van Dam *et al.*, 5) that casein prepared by supercentrifugation is detrimental to loaf volume, but are not in agreement with their other observation that the corresponding supercentrifuge whey has no adverse effect.

The first step in attempting to assign the effects of skimmilk on bread to any of its constituents has usually been to fractionate the skimmilk into casein and whey and to incorporate these separately into dough. Three methods of removing casein, involving respectively acid coagulation at the isoelectric point, rennet coagulation, and supercentrifugation, have been employed by various workers. The fractions obtained by these three methods are not of identical composition. Casein exists in milk in the form of particles containing, in addition to the protein itself, considerable amounts of calcium and phosphate and a little magnesium and citrate. In acid coagulation, free casein is precipitated and the associated salts are transferred to the whey. Rennet coagulation, on the other hand, involves no change

[1] Manuscript received September 18, 1953. Paper No. 3048, Scientific Journal Series, Minnesota Agricultural Experiment Station. The data in this paper constitute part of a thesis presented to the Graduate School of the University of Minnesota by A. L. Gordon in partial fulfillment of the requirements for the Ph.D. degree, August 1952.

[2] Present address: A. E. Staley and Co., Decatur, Illinois.

[3] Professors of Agricultural Biochemistry, University of Minnesota, St. Paul.

in pH and the associated salts remain with the casein. Likewise, when the casein particles are removed by high-speed centrifugation the associated salts remain with the particles, but the separation is usually not complete, some casein particles remaining in the whey.

Both the casein and whey fractions obtained by acid coagulation have been shown to depress loaf volume when incorporated into bread doughs (2, 3). Harland *et al.* (1) reported significant depressions by acid whey but not by acid-precipitated casein. Rennet whey also depresses loaf volume (1, 2), but rennet-clotted casein does not seem to have been investigated for baking quality. In contrast to these observations, van Dam *et al.* (5) have reported that casein prepared from skimmilk by supercentrifuging is detrimental to loaf volume, whereas the corresponding supercentrifuge whey had no adverse effect. These workers also found that calcium caseinate prepared in the laboratory improved loaf volume, a deleterious effect being exerted only when the colloidal calcium phosphate was also present. Heeres and Meijknecht (2), on the other hand, observed depressions of loaf volume with calcium caseinate, sodium caseinate, and a water dispersion of casein.

These discrepancies in the literature prompted the present study of the effects on breadmaking of caseins and wheys prepared by acid and rennet coagulation and supercentrifugation. In addition some attention was devoted to caseinate sols of controlled composition in an effort to check the contentions of van Dam *et al.* (5).

Materials and Methods

All milk fractions employed were prepared from unheated skimmilk from the University Dairy. Supercentrifugation was accomplished in the batch bowl of a Sharples Supercentrifuge operating at 40,000 r.p.m. for 30 minutes. This procedure resulted in a fairly good separation of casein and whey. Approximately 10% of the casein remained in the whey, a result comparable to that reported by van Dam *et al.* (5). The casein fraction was removed from the wall of the centrifuge bowl with a large spatula and triturated in distilled water with a mortar and pestle to produce a uniform sol. The whey fraction was strained through cotton to remove traces of fat which were present.

Acid precipitation was performed by adjusting skimmilk to pH 4.6 with *N* hydrochloric acid solution. After the casein was filtered off, both a water suspension of casein and the whey were adjusted to pH 6.6 with *N* sodium hydroxide solution.

Rennet casein and whey were prepared by treating 600 ml. of skim-

milk with 3 ml. of a diluted (1:10) commercial rennet extract (Chr. Hansen's Laboratories, Little Falls, New York) and holding at 35°C. for 20 minutes and subsequently at room temperature for 1 hour to expel the whey from the casein. The casein clot was homogenized in distilled water with a Waring Blendor for 5 to 10 minutes to produce the finely divided suspension which was used in baking studies.

Calcium caseinate sols were prepared by titrating a water suspension of acid-precipitated casein with a saturated solution of calcium hydroxide until the desired amount of calcium was added. The pH was then adjusted to 6.5 with a dilute solution of hydrochloric acid or sodium hydroxide.

To evaluate the effects of these fractions in breadmaking, experimental baking tests were conducted employing the modification of the A.A.C.C. basic formula and procedure described by Larsen *et al.* (3). The respective fractions were used in quantities which would be equivalent to the amounts present if 6% nonfat milk solids were included in the formula; the relative concentrations of the casein and whey fractions in skimmilk were based upon determinations of nitrogen distribution by the Rowland method (4). The fractions were added as aqueous dispersions to which the yeast, salt, and sugar were added; sufficient additional water was used to give the desired absorption (58% in some tests and 62% in others). No shortening was used.

Results

Casein and Wheys. Raw skimmilk and the casein and whey fractions prepared therefrom all depressed the loaf volume of bread below that of the controls. As shown in Fig. 1, however, the casein fractions were more deleterious than whey in their baking action.

A second series of baking tests was performed by two collaborating bakers (with omission of the rennet fractions) using an absorption of 62%. The results shown in Fig. 2 substantially agree with those of the first series.

Sodium and Calcium Caseinate Sols. In the above experiments acid-precipitated casein was incorporated into dough in the form of sodium caseinate, but the rennet and supercentrifuged caseins contained considerable calcium and phosphate. The fact that all three preparations behaved similarly is evidence against any specific action of calcium or phosphate. However, as a further test, a series of bakes was made with sodium caseinate and three calcium caseinate sols containing different levels of calcium. A calcium phosphate suspension containing 125 mg. each of calcium and phosphate (as PO_4) per 100 ml. and hav-

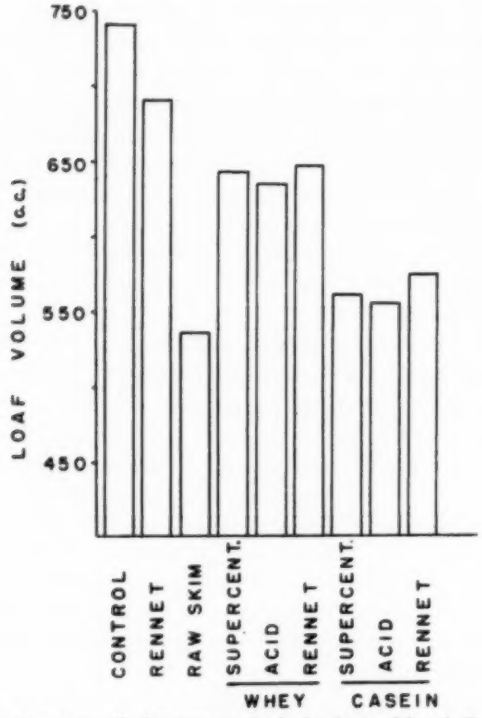

Fig. 1. Effect of various milk fractions on the loaf volume of bread. Doughs were made with 58% absorption (flour basis). (In the bake designated "rennet," 0.5 ml. of a 1:10 dilution of commercial rennet extract was added per 200 g. of flour.)
Each value represents the average of duplicates. The standard error of a single determination in this series was 12.7 cc.

ing a pH of 6.0 was also included in this series at two levels in the absence of casein and at one level with casein. The results, shown in Table I, indicate a similar behavior for casein regardless of the manner in which it is dispersed. Only a slight depression was produced by the calcium phosphate suspension.

Discussion

The findings of van Dam *et al.* (5) that casein prepared by supercentrifuging depresses the loaf volume of bread were confirmed. However, it was also demonstrated that bread baked with supercentrifuged whey as an adjunct was consistently 10 to 15% lower in loaf volume than the controls. This agrees with results previously reported for acid and rennet whey (1, 2, 3) but not with those of van Dam *et al.* (5) for supercentrifuged whey.

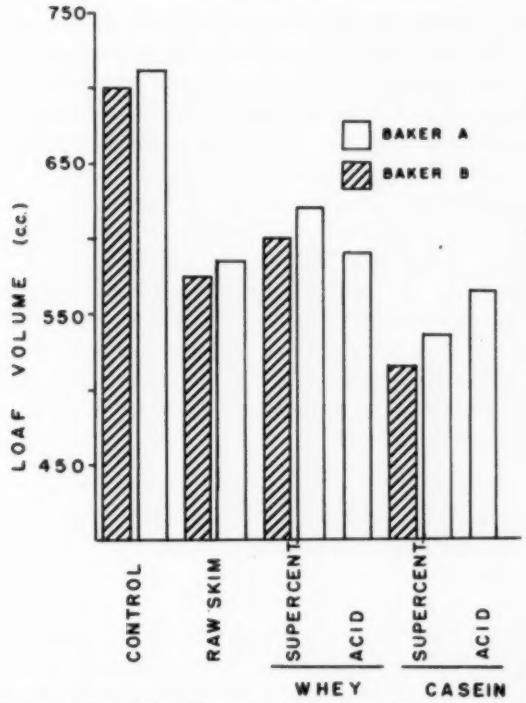

Fig. 2. Effect of various milk fractions on the loaf volume of bread. Baking tests were conducted by two bakers, employing 62% absorption (flour basis).
Each value represents the average of duplicates. The standard error of a single determination in this series was 13.3 cc.

TABLE I

EFFECT OF CASEINATE SOLS AND OF SALT SOLUTIONS ON LOAF VOLUME

Dough Supplement	Calcium Hydroxide per 3 g. Casein	pH of Supplement	Mean Loaf Volume[1]
	mg.		*cc.*
None—control	. .	6.0	772
Sodium caseinate[2]	0	6.0	743
Calcium caseinate[2]	42	. .	788
Calcium caseinate[2]	84	6.6	618
Calcium caseinate[2]	182	6.5	628
Acid casein[2] in 80 ml. salt solution		6.5	625
40 ml. salt solution[3]		6.5	613
80 ml. salt solution[3]		6.0	623

[1] Each value represents the average of duplicates. The standard error of a single determination in this series was 11.1 cc.
[2] Three g. of casein per 200 g. flour.
[3] Solution consisted of 0.460 g. $CaCl_2 \cdot 2H_2O$, 0.130 g. Na_2HPO_4, and 0.124 g. $NaH_2PO_4 \cdot H_2O$ per 100 ml. Thus it contained 125 mg. each of calcium and phosphate (as PO_4) per 100 ml. A fine flocculent precipitate formed in this mixture. It was mixed thoroughly before use.

In general, the method of fractionating milk into casein and whey components had very little influence on baking behavior. Acid-precipitated casein was slightly superior to rennet-clotted casein and supercentrifuged casein in baking behavior. This may be due to a loss of milk salts associated with the casein component in milk which were removed by acidifying milk to a pH of 4.6.

The contention of van Dam *et al.* (5) that calcium caseinate improves loaf volume whereas sols containing both calcium caseinate and phosphate are depressants was not confirmed. In all cases in the present study calcium caseinate sols depressed loaf volume regardless of whether they contained phosphate or not. Possibly one reason for the discrepancy is that the pH of the calcium caseinate sols in the present experiments was maintained at a constant value of 6.5, whereas in van Dam's studies it varied from 5.7 to 10.4 depending on the concentration of calcium present.

Acknowledgment

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PURIFICATION OF ALPHA-AMYLASE FROM SUBMERGED CULTURES OF ASPERGILLUS ORYZAE[1]

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ABSTRACT

An adsorption procedure has been developed which allows the alpha-amylase from submerged cultures of *Aspergillus oryzae* to be concentrated from 15- to 37-fold by adsorption of the enzyme on a portion of the mold mycelium followed by elution with a borate buffer. This procedure at the same time eliminates over 99% of the proteinases, 93% of the limit dextrinase, and 87% of the Kjeldahl nitrogen present in the original fermentation liquor.

Mycelium of *Aspergillus oryzae* failed to adsorb the alpha-amylase from *Aspergillus niger* and malt extract, indicating that either these alpha-amylases must differ somewhat in structure, or certain interfering substances are present.

A great deal of work has been carried out to test the feasibility of using fungal alpha-amylase for the diastating of flour. Green (3), Kneen and Sandstedt (8), Miller and Johnson (9), Johnson and Miller (7), and Harrel, Lincoln, and Gunderson (4) have suggested that fungal amylase might be suitable in baking as a replacement for malted wheat flour. Some of the advantages claimed for fungal amylase over malted wheat and barley flour are reduction of "buckiness" in doughs by increasing their mobility during fermentation, control of dough consistency, improvement of loaf volume, increase in softness of crumb, and elimination of sticky crumb due to an excess of supplement. Fungal supplements also make possible the production of a lighter colored flour. However, the proteolytic activity associated with fungal cultures has been found to be quite variable, and since excessive dosages of proteinases have been found to be detrimental by many investigators (2, 5, 6, 9, 13), any method used to produce a diastating enzyme from fungi must be designed to control the proteolytic activity as well. Since the main disadvantages in the use of fungal enzymes for the diastating of flour are their cost of production and the high concentration of proteolytic enzymes associated with the amylase, this investigation has been directed toward a less expensive method of preparation which at the same time would eliminate a great deal of the undesirable enzyme activity.

Yuzo Tokuoka (17, 18) first reported that the alpha-amylase from

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moto-koji could be adsorbed by the residue left after the enzyme had been extracted. He prepared koji by growing a culture of *Aspergillus oryzae* on steamed rice. The alpha-amylase was extracted with water and a 1% sodium chloride solution. When a small amount of the extracted residue was added to this extract and 95% ethanol added until the alcohol concentration reached 40%, Tokuoka found the alpha-amylase was almost quantitatively adsorbed on the residue. He thought that the rice somehow adsorbed the enzyme, stating that "although takadiastase could not be adsorbed by starch powder or oryzinin it is markedly adsorbed by steamed rice."

Schwimmer and Balls (15, 16) used 40% ethanol and wheat starch to adsorb alpha-amylase from malt and likewise obtained almost quantitative removal of the enzyme. They also have shown that different starches have a profound effect on the adsorption of alpha-amylase. In view of these facts, and the fact that we are unable to get any adsorption on mycelium in the presence of 1% sodium chloride, the authors have concluded that the active adsorbent of the alpha-amylase in the Tokuoka experiment was the rice starch present in his moto-koji and not the small amount of mycelium of *Aspergillus oryzae*.

Materials and Methods

Production of Fungal Amylase Cultures. Stock cultures of molds were maintained on a medium containing corn starch (1.5%), potassium nitrate (0.4%), calcium carbonate (0.1%), primary potassium phosphate (0.05%), magnesium sulfate (0.025%), potassium chloride (0.02%), iron, zinc, and copper (5 p.p.m.), and agar (2%). After the cultures were well sporulated they were stored at 4°C. until used. The original cultures were covered with sterilized paraffin oil and kept at 4°C.

The inoculum was prepared by taking a 48-hour slant culture prepared in the same manner as the stock cultures and transferring a loopful of mold to 200 ml. of the same medium, less agar, contained in a 1-liter wide-mouth Erlenmeyer flask.

One of the flasks, after 48 hours' incubation in a reciprocal shaking machine, was used to inoculate 10 l. of the same medium in a large Pyrex bottle equipped with an aerator made by attaching a canvas bag to the end of the air delivery tube, through which sterile air was passed at a rate of one-third volume of air per volume of medium per minute. The cultures were aerated at 30°C. until enzyme production was maximum. This value was reached in 3 to 6 days.

During the latter part of this investigation the potassium nitrate was replaced by urea.

Alpha-Amylase Activity. The alpha-amylase was determined by Olson, Evans, and Dickson's (11, 12) modification of the Sandstedt, Kneen, and Blish (14) procedure. However, since *Aspergillus oryzae* does not produce any beta-amylase, the soluble starch substrate was not treated with beta-amylase. Our unit of alpha-amylase is the amount of enzyme which dextrinizes 1 g. of Lintner's soluble starch in one hour at 30°C. (One alpha-amylase unit on starch which is not treated with beta-amylase equals 8.8 units on beta-treated starch under our conditions.)

Limit Dextrinase. The limit dextrinase activity was determined by the method of Back, Stark, and Scalf (1). The unit of measurement is expressed as percent hydrolysis of limit dextrin to fermentable sugar in 1 hour, at 30°C., by 1 ml. of enzyme solution.

Proteolytic Activity. The proteolytic activity was measured by the method of Northrop (10). The pH of the gelatin was held at 7.5 with a phosphate buffer. A change of 1% per minute in specific viscosity of a standard gelatin solution is defined as one unit of proteolytic activity.

Adsorption and Elution Procedure. The culture was grown under forced aeration on a medium containing starch, a nitrogen source, and essential minerals. When amylolytic activity was maximum, the mycelium was removed from the fermentation liquor by filtration, after adjustment of the pH to 8.5 with dilute sodium hydroxide. Since the cultures always finished at pH 7.8–8.2, this took very little alkali, but it was necessary in order to get complete separation of the enzyme from the mycelium. Approximately one-fifth of the mycelium (5 g. of mycelium per l., containing 85% moisture) was washed and then resuspended into the culture filtrate. The pH of the filtrate was adjusted to 5.0 with glacial acetic acid, the temperature adjusted to 37°C., and the entire mixture agitated gently for 1 hour. The mycelium was then removed by filtration, washed with a little water which had been adjusted to pH 5, and resuspended in a minimum amount of solution (1/20–1/40 the original volume of fermentation) containing 1% sodium chloride dissolved in a solution consisting of 25% by volume of borate buffer and 75% water. The borate buffer was prepared by mixing 50 ml. of 0.2*M* potassium chloride, 50 ml. of 0.2*M* boric acid, and 8.5 ml. of 0.2*M* sodium hydroxide and making up to a total volume of 200 ml. The pH of the elution liquid was then adjusted to 8.5 with sodium hydroxide. This solution was allowed to stand for 0.5 hour with gentle agitation, then the mycelium was removed by filtration, leaving a filtrate in which the alpha-amylase had been concentrated from 15- to 37-fold with the removal

of substantial amounts of limit dextrinase and proteolytic activity. In later experiments the borate buffer and water was replaced with a 2% sodium bicarbonate solution which was equally satisfactory.

Results and Discussion

Effect of pH on Adsorption. Preliminary experiments had indicated that mycelium of *Aspergillus oryzae* would adsorb its alpha-amylase from the culture liquor. Since maximum adsorption should be observed at the pH which causes the greatest differential in the charge carried by the mycelium and enzyme, a series of experiments was made to determine the optimum pH for adsorption by adjusting the pH of culture liquor with glacial acetic acid.

pH of Culture	*Amylase Adsorbed* %
4.50	66
4.75	66
5.00	93
5.50	72
6.00	70

From the above it is apparent that the most favorable pH is 5.

Effect of Temperature on Adsorption. Preliminary work indicated that the temperature of the fermentation liquor also had a very definite influence on the rate of adsorption. To determine the optimum value, a series was run by adjusting the culture liquor to pH 5 and agitating in water bath for 1 hour at various temperatures.

Temperature, °C.	*Amylase Adsorbed* %
25	77
30	86
35	97
37	98
40	96

These results indicated that any temperature between 35° and 40°C. was satisfactory.

Effect of Concentration of Mycelium on Adsorption. In the preliminary work it was observed that the addition of a higher concentration of mycelium increased the amount of amylase recovered. However, excessive amounts of mycelium limit the final concentrations which can be obtained. Concentrations of more than 15 g. of mycelium per l. resulted in a pastelike consistency in the elution liquid if concentrations of 20-fold were being attempted. For this reason our results were obtained on the basis of 5 g. of mycelium per liter. However, to determine the results of using higher concentrations a

series of tests was run on one exceptionally poor batch of mycelium. These results are expressed below.

Mycelium (85% Moisture) g./l.	*Recovery of Alpha-Amylase* %
5	60
10	66
15	79
20	87
25	89

From these results it can be observed that the recovery increases with increasing amounts of mycelium.

Effect of Salt Concentration on Adsorption. Although the elution of the enzyme did not offer a problem (elutions of 90 to 100% were obtained regularly), the adsorptions were not always satisfactory, as indicated in the following table.

Test No.	*Times Concentrated*	*Recovery* %
10	15.0	75
11	16.0	80
12	19.0	60
13	37.0	75
14	32.0	74
15	15.5	41
16	22.3	46
17	26.0	52
18	31.0	62
19	25.0	90
20	34.0	78
21	35.0	85

Since the salt concentration was found to be very important in elution, it appeared reasonable that the excess salt present in the medium might be responsible for the erratic results observed. The chief source of salt in the culture liquor was the residual potassium bicarbonate which results from the potassium nitrate used as a nitrogen source. If this was true, replacing the potassium nitrate with a nitrogen source which would not leave a salt residue should remove this obstacle. One way this could be done was by feeding in nitric acid during the fermentation, being careful not to lower the pH below 4. This was tried and found quite satisfactory. The other possibility was to replace potassium nitrate with a nitrogen source, such as urea, which would leave no residue. This was tried and found extremely satisfactory, as the following table indicates.

The average adsorption of the first nine runs using urea gave an average recovery of 90.8%.

Test No.	*Times Concentrated*	*Recovery* %
36	24	95.2
37	24	98.7
38	22	91.6
40	18	81.3
41	23	91.0
42	18	85.0
43	15	82.5
44	21	94.0
45	18	98.0

Concentration and Fractionation of Alpha-Amylase by Adsorption. With the fact established that alpha-amylase could be easily concentrated by this method, it was desirable to see if, at the same time, the alpha-amylase was separated from other enzyme systems. To test this

TABLE I

CONCENTRATION AND FRACTIONATION OF ALPHA-AMYLASE FROM LIMIT DEXTRINASE, KJELDAHL NITROGEN, AND PROTEOLYTIC ACTIVITY BY MYCELIUM ADSORPTION

	Fractions				
	1	2	3	4	5
	Mold Filtrate as Grown, 20 l.	Filtrate from (1) after Mycelium Adsorption	Elution Liquid from Mycelium Adsorption 350 ml.	Filtrate from (3) after Second Mycelium Adsorption	Elution Liquid from Second Adsorption
Amylase units/ml.	1.50	0.22	43.00	0.32	110.00
Percentage of original amylase	100.00	14.70	78.50	0.59	73.50
Concentration by activity	....	....	29.00	....	73.00
Kjeldahl nitrogen mg./ml.	0.1475	0.1380	0.855[1]	0.255	1.80[1]
Percentage of original Kjeldahl nitrogen	100.00	93.50	15.90	4.70	12.20
Limit dextrinase hydrolysis/ml.	2.01	1.90	5.67	4.50	12.50
Percentage of original dextrinase	100.00	94.50	7.75	2.24	6.20
Proteolytic activity units	3.00	1.99	1.03	0.30	1.72
Percentage of original proteolytic activity	100.00	66.40	0.94[2]	0.27	0.57

[1] Probably some additional nitrogen released from mycelium under conditions of elution.
[2] Apparently, either part of the proteolytic enzyme is adsorbed but fails to elute under the conditions used, or it may be inactivated by the elution treatment.

procedure, 20 l. of mold filtrate was concentrated to 550 ml. in one adsorption and this filtrate was further concentrated by a second adsorption to a volume of 200 ml. with a total concentration of 100 times by volume. The alpha-amylase activity, limit dextrinase activity, Kjeldahl nitrogen, and proteolytic activity were followed through these purification steps. The results are presented in Table I.

It can be observed that the amount of the enzymes removed by the elution liquid and that found in the final concentrate do not add up to 100% in each case. This is due to several factors, the most important being that the enzymes may not be completely removed by elution under the conditions of the experiment, and secondly, some of these methods of analysis are not accurate to more than 3 to 5%.

From these results it can be observed that a second concentration by this adsorption procedure was possible. However, it was discovered that it was necessary to first dilute the solution so that the salt concentration was less than 0.5%, or to dialyze it free of salts before the second adsorption. This adsorption procedure was found to eliminate 93.8% of the limit dextrinase activity, 87.8% of the Kjeldahl nitrogen, and 99.4% of the proteolytic activity.

Concentration of Adsorption Filtrate by Vacuum-Distillation. Since the filtrates obtained from this adsorption procedure are low in salt concentration (approximately 1%), they can be readily concentrated by vacuum distillation with no loss in activity. We have readily obtained concentrations of 465 times that of the original culture filtrate by a combination of adsorption and vacuum distillation with over-all recoveries of 73.5%.

Selectivity of the Adsorption of Alpha-Amylase on the Mycelium of Aspergillus oryzae. This adsorption process appeared to be very selective, as the mycelium from *Aspergillus oryzae,* when run under the same conditions as were found to be optimum for the adsorption of its own alpha-amylase, failed completely to adsorb the alpha-amylase from malt extract and *Aspergillus niger,* regardless of whether or not these enzyme solutions were first dialyzed to free them of salts.

In order to test the possibility of this technic being applicable only to the strain of *Aspergillus oryzae* we were using, two cultures, NRRL 454 and NRRL 694, were obtained from the Northern Regional Research Laboratory. Used under optimum conditions for our own culture, they gave adsorption of 79% and 59%, respectively. Although these values are a little low, probably the optimum conditions for these cultures may vary slightly from ours. The adsorbed enzyme from both cultures was 90% eluted by our elution technic.

The fact that alpha-amylase from *Aspergillus niger* and from malt failed to adsorb on mycelium of *Aspergillus oryzae* warrants further investigation. This would indicate that either these enzymes have a structure slightly different from that of the alpha-amylase of *Aspergillus oryzae*, or certain substances are present which may interfere with their adsorption.

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BAKING BEHAVIOR AND OXIDATION REQUIREMENTS OF SOY FLOUR. I. COMMERCIAL FULL-FAT SOY FLOURS[1,2]

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ABSTRACT

A survey of the baking behavior of presently available full-fat soy flours, when used at a 5% level based on the wheat flour, was made to determine their effect on dough and bread properties. The results show that when potassium bromate is used in the range of 1.0 to 3.0 mg. % on wheat flour basis, the deleterious effects usually attributed to soy flour are almost entirely absent. Dough properties are satisfactory and loaf volume nearly equivalent to that of the basic loaf; some change in crumb color is detectable. The best grades of soy flour have a nitrogen solubility in water in the range of 35 to 45%. Water absorption of the dough was increased 0.8% for each % of soy flour added.

During recent years there has been considerable interest in the fortification of commonly used low-cost foods with protein, minerals, and vitamins as a means of bettering the nutritional status of people in all economic brackets. The wisdom of such fortification with vitamins and minerals has been very well demonstrated in the 1944 and 1948 medical surveys of nutrition in Newfoundland (1,2) and by the recent survey, done at the hospitals of the State University of Iowa, of the dietary habits of pregnant women. In this survey, Jeans, Smith, and Stearns (9) reported that 59% of the women included in their survey had a protein intake less than 75% of the recommended protein allowance of 85 g. daily.

The American miller and the American baker have contributed to the welfare of the people through the bread-enrichment program which during the past 10 years has aided in the more general attainment of optimum vitamin and mineral intake. A food so universal and used in such quantities as bread might easily carry its load of other nutrients in proportion to the calories it furnishes.

It is common knowledge that the wheat proteins are deficient in lysine and valine, and Light and Frey (10) have demonstrated that the supplementary value of other proteins for wheat protein results from the addition of these amino acids. Soy flour, in addition to raising the total protein content of the bread, has a specific effect because full-fat

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soy flour (44% protein) contains 12 times as much lysine and 4.3 times as much valine as a wheat flour of 12% protein. With defatted soy flour (50% protein) the increase is even greater, there being 13.6 times as much lysine and 4.9 times as much valine in the soy flour as in a 12% protein white wheat flour. Lysine and valine values were calculated from the data of Block and Bolling (5).

Finney (7), working with a 15% protein spring wheat flour and defatted soy flour, stated that "excellent bread can be made with blends containing up to 8% soy flour with hard red spring wheat flour, the quality of the bread as measured by loaf volume and crumb grain being even better than for wheat flour alone provided the quantity of potassium bromate used in the baking formula is increased according to the amount of soy flour used." Bayfield and Swanson (3) pointed out that expeller-type soy flours showed inferior baking characteristics. It should be realized that the processing methods used for this material cannot yield a flour of uniform quality. An excellent and comprehensive study of the functional properties of soy flour as a bread ingredient was published by Bohn and Favor (6). Although these investigators state that "bread made with soy flour is more compressible and the rate of staling is slower than for bread produced under similar conditions from wheat flour alone," there seems to be a prejudice in the baking industry based upon long-past unfavorable experiences. However, present-day flours show much improvement over former products.

The full benefits of proven nutritional advantages obtained by the use of soy flour as a supplement to wheat flour in bread will be attained only when soy flour is accepted by the American baker at wholesale and retail levels. This acceptance by the baker can be achieved by making it possible for him to obtain, from any processor, soy flour comparable in uniformity with his wheat flour purchases. It is equally important for the baker to have at hand information on the correct use of soy flours.

The present investigation was undertaken to determine by a baking survey whether previous objections to soy flours by bakers are applicable to present products which are commercially available. The purpose of this paper is to present the data on soy flour obtained through the survey and to emphasize some of the requirements for the successful use of this valuable ingredient.

Materials and Methods

A sample of full-fat flour was obtained each month for 6 months from the production of each of four manufacturers. The flours, as

received, were immediately placed in friction-top cans and stored at 34°F. until they were used.

The wheat flour used was a commercially milled baker's patent with 0.44% ash and a protein content of 11.4%, both expressed on a 14.0% moisture basis.

The wheat flour standard was baked with optimum bromate, 5% sugar, 2% yeast, 2% salt, and 4% shortening. Water absorption was adjusted by the operator to yield optimum dough consistency. Optimum mixing time with a straight dough procedure and a 3-hour fermentation at 30°C. were employed. The first punch was performed manually, and doughs were scaled to 530 g. at the second punch and punched on a National dough sheeter. Molding was accomplished by sheeting on a National dough sheeter, hand curling and molding on a National molder. Baking time was 30 minutes at 220°C. in a rotary-hearth electrically heated oven.

For a comparison of the soy flour with milk, some doughs were made by adding 4% nonfat dry milk solids, based on the flour weight, to the standard and baking at optimum absorption and optimum bromate content according to the procedure outlined above.

The soy flours were added, on a fat-free basis, to the wheat flour standard at a 5% level based on the amount of wheat flour, and shortening was decreased by the amount of fat in the added soy flour.

Each soy flour was baked at various bromate levels and at optimum absorption, by the same procedure used for the standard wheat flour.

Doughs were mixed to yield duplicate loaves at each bromate level and duplicate mixes were made the same day. When the different samples of soy flour were compared, under optimum conditions for each flour, the bakes were repeated on different days, with duplicate mixes of duplicate loaves baked each day. Thus, in the bromate series, each value represents an average of four loaves, and in the flour comparison series each value is obtained by averaging loaf volumes of eight loaves. All loaves were allowed to cool for 1 hour at room temperature before determination of loaf volume in a National volumeter. After determination of loaf volume, the loaves were stored overnight in tin-lined drawers.

Protein solubility of the soy flours was determined by shaking 2.5-g. aliquots, plus 100 ml. water, in a 250-ml. centrifuge bottle, on a commercial reciprocating shaker for 30 minutes, centrifuging, and determining nitrogen in the supernatant liquor by the Kjeldahl method. This value is expressed as percentage of the total nitrogen of the meal.

Results and Discussion

In order to attain comparable dough consistency at the time of panning, it was necessary to mix all doughs containing full-fat soy flour to a slightly stiffer consistency than that of the basic wheat flour doughs. This indicates a slight slacking-off during fermentation of all doughs containing full-fat soy flour. In addition, all doughs contain-

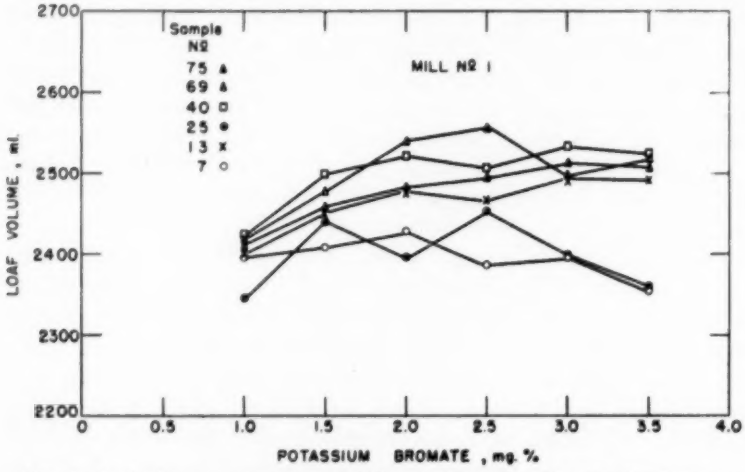

Fig. 1. Loaf-volume responses from addition of increments of postassium bromate to doughs containing 5% full-fat soy flours obtained from Mill No. 1 over a 6-month period.

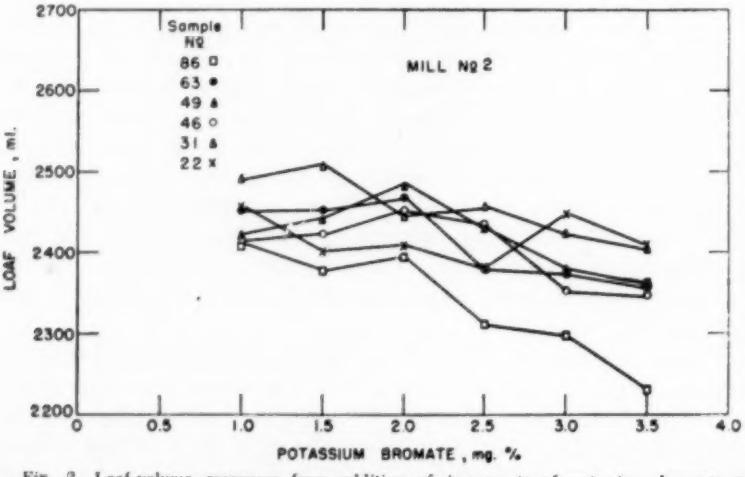

Fig. 2. Loaf-volume responses from addition of increments of potassium bromate to doughs containing 5% full-fat soy flours obtained from Mill No. 2 over a 6-month period.

ing soy flour were slightly stickier at mixing than the basic wheat flour doughs, but this condition did not persist after fermentation, particularly at optimum bromate levels.

There was no apparent change in mixing time with incorporation of 5% full-fat soy flour, and the increase in water absorption due to the added soy flour was slightly under 1% for each percent of soy flour added on a full-fat basis. This is in agreement with the absorption increase determined by Finney (7) and promulgated by the trade.

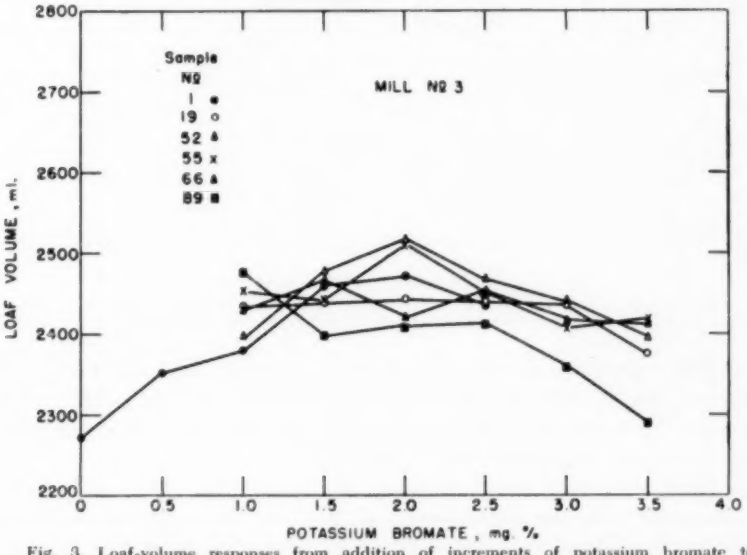

Fig. 3. Loaf-volume responses from addition of increments of potassium bromate to doughs containing 5% full-fat soy flours obtained from Mill No. 3 over a 6-month period.

Loaf-volume response to potassium bromate is shown in Figs. 1 through 4. These graphs show the wide variations in optimum bromate requirement and in oxidation tolerance of the monthly samples from each manufacturer as well as the differences between full-fat flours from different manufacturers.

A loaf-volume comparison of all the full-fat soy flours with the basic wheat flour and with the wheat flour plus 4% nonfat dry milk solids is shown by the bar graph in Fig. 5. The loaf volumes shown are not the maximum attainable, but are rather the volumes obtained when each flour was baked at that oxidation level which represents the optimum over-all score, with internal and external characteristics of the bread and with dough-handling character considered as well as loaf volume.

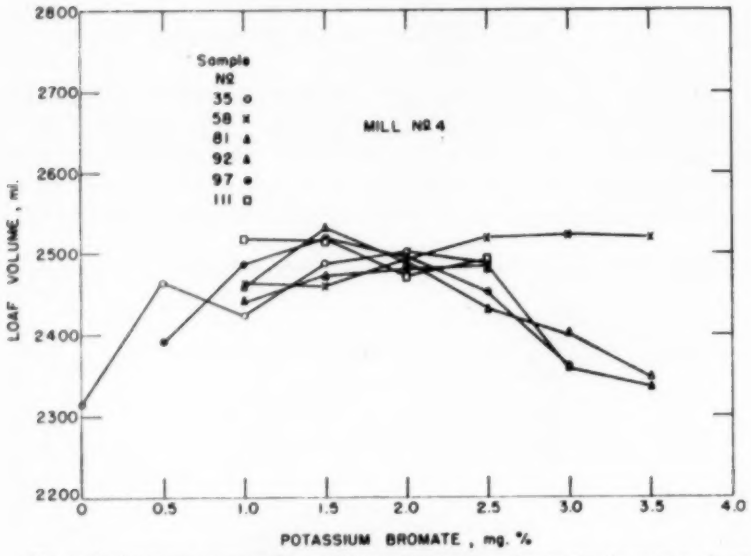

Fig. 4. Loaf-volume responses from addition of increments of potassium bromate to doughs containing 5% full-fat soy flours obtained from Mill No. 4 over a 6-month period.

Although loaf volume of all but one of the soy flours was less than the average volume for the basic wheat flour and the milk-supplemented flour, the differences in loaf volume were not sufficiently great in many cases to be considered important.

Dough-handling character upon the addition of soy flour varied a great deal with the different soy flours over the range of oxidation used. These differences between flours were considerably minimized by baking at the optimum oxidation level.

There seemed to be little correlation between loaf volumes and dough-handling characteristics. The flours which seemingly had the best tolerance to oxidation and produced doughs most nearly like those obtained with nonfat dry milk solids did not necessarily produce the best loaf volumes. In fact, sample 63, which had the lowest loaf volume, gave doughs with excellent handling character, whereas sample 7, which had good loaf volume, yielded doughs which were "dead" and had poor handling character. Conversely, sample 31 with very low loaf volume was baked from doughs which were "dead," but sample 58 with good handling character yielded loaves of good volume.

Values for protein solubility are given in Fig. 5. A comparison of these values with those plotted by Belter and Smith (4) against time

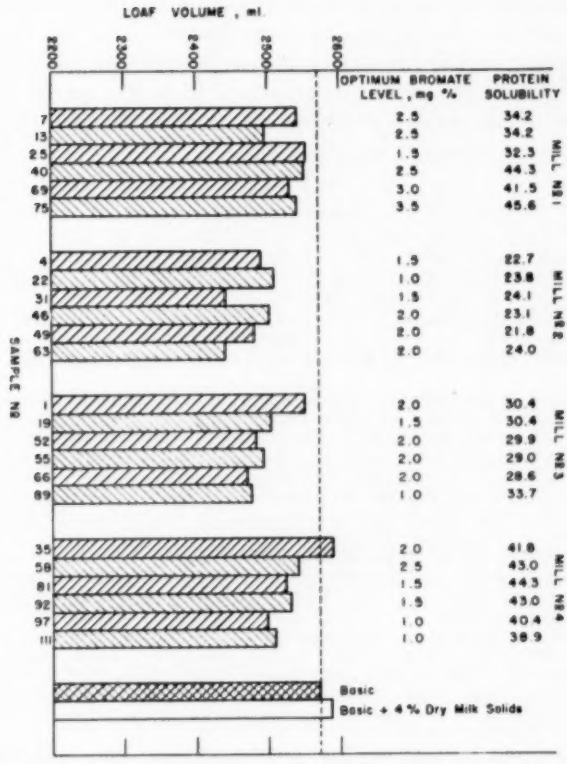

Fig. 5. Comparisons of the loaf volumes of breads containing 5% of each of 24 full-fat soy flours, baked at the indicated optimum potassium bromate level, with those of bread baked from the basic wheat flour alone and bread with 4% nonfat dry milk solids added. Also shown are the protein solubilities of the full-fat soy flours.

of heat treatment shows that the heat treatment applied to the full-fat flours is most certainly very light as compared with the treatment normally given meals used for feed. There was, however, no correlation between the protein solubility values and loaf volume or dough-handling character. It must be concluded, therefore, that the protein solubility test cannot, with our present knowledge, be used to predict baking quality.

The values for optimum bromate level for best bread production tabulated on Fig. 5 indicate that care must always be exercised by the baker to use levels of oxidizing agent higher than normal when soy flour is incorporated in the dough, and that this level may vary considerably with different lots of full-fat soy flour obtained from any

one manufacturer. This is in accord with statements in the papers of Bohn and Favor (6), Finney (7), and Finney *et al.* (8).

Experience in this laboratory has indicated that with the use of optimal bromate the difficulties encountered in the use of full-fat soy flours now commercially available are minimized to a point where they are not of practical significance.

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BAKING BEHAVIOR AND OXIDATION REQUIREMENTS OF SOY FLOUR. II. COMMERCIAL DEFATTED SOY FLOURS[1,2]

C. W. Ofelt, Allan K. Smith,[3] and James M. Mills[4]

ABSTRACT

A survey of the baking behavior of present-day defatted soy flours was made to determine their effect on dough and bread properties. It was found that with the proper amount of oxidation the use of soy flour up to 5% does not significantly impair loaf volume, crumb character, or dough handling properties. Nitrogen solubilities of defatted soy flours are in the range of 50 to 60% and the bromate requirements 3.0 to 4.0 mg. %, based on the weight of the wheat flour where 5% soy flour is used. An increase in dough absorption of 1% for each % of soy flour was found desirable.

There is ample evidence in the literature (2,4) that wheat proteins are deficient in the two essential amino acids, lysine and valine, and that this deficiency can be overcome for all practical purposes by a judicious supplemetation with soy flour. It has also been reported by Ofelt, Smith, Evans, and Moser (5) that the flavor of bread made with the addition of 5% defatted soy flour was not differentiated, by a group representative of the consuming public, from the flavor of bread made with 4% nonfat dry milk solids.

Bohn and Favor (3) made an excellent study of the effects of soy flour as a bread ingredient and Ofelt, Smith, and Derges (6) have made a survey of currently available commercial full-fat soy flours to determine their baking behavior and oxidation requirements. The purpose of the present study is to increase the scope of information on commercial flours by extending the survey to include the defatted flours now available to the food industry.

Materials and Methods

A sample of defatted soy flour was obtained each month for 5 months from the production of each of four manufacturers. The flours, as received, were immediately placed in friction-top cans and stored at 34°F. until they were used.

The wheat flour used was an unbleached commercially milled baker's patent with 0.44% ash and a protein content of 11.4%. This

[1] Manuscript received May 14, 1953.
[2] Presented at the Annual Meeting of the American Association of Cereal Chemists, Buffalo, New York, May 25-28, 1953.
[3] Northern Regional Research Laboratory, Peoria, Illinois, one of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.
[4] Present address: Kansas Milling Co., Wichita, Kansas.

flour had weak characteristics and because of its sensitivity was excellent for studying the use of added ingredients.

Baking methods and the method of determining protein solubility were the same as those previously reported (6).

Results and Discussion

It was necessary to mix all doughs containing defatted soy flour to a slightly stiffer consistency than the basic wheat flour doughs, if comparable dough consistency was to be attained at the time of panning. This indicates a slight slacking off during fermentation of all doughs containing the defatted soy flour. In addition, all doughs containing the defatted soy flour were slightly stickier at the mixer than the basic wheat flour doughs. In most doughs, particularly at optimum bromate levels, this stickiness did not persist through the fermentation period. With some flours, however, this dough stickiness was not overcome by fermentation or oxidizing agents.

There was no apparent change in mixing time with incorporation of 5% defatted soy flour, and the increase in water absorption due to the added soy was approximately 1% for each percent of soy flour added. This is slightly less than values to be found in the literature and promulgated by the trade.

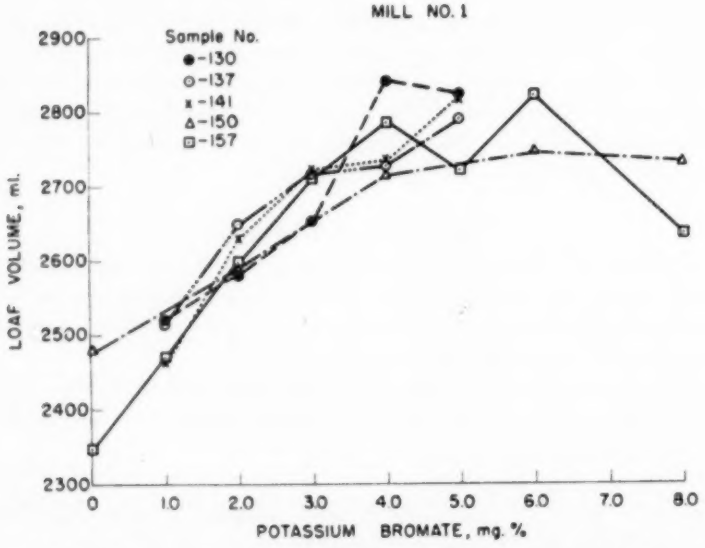

Fig. 1. Loaf-volume responses from addition of increments of potassium bromate to doughs containing 5% defatted soy flours obtained from Mill No. 1 over a 3-month period.

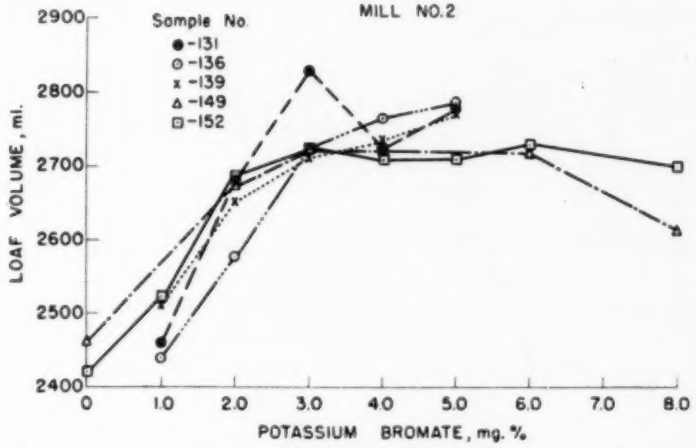

Fig. 2. Loaf-volume responses from addition of increments of potassium bromate to doughs containing 5% defatted soy flours obtained from Mill No. 2 over a 5-month period.

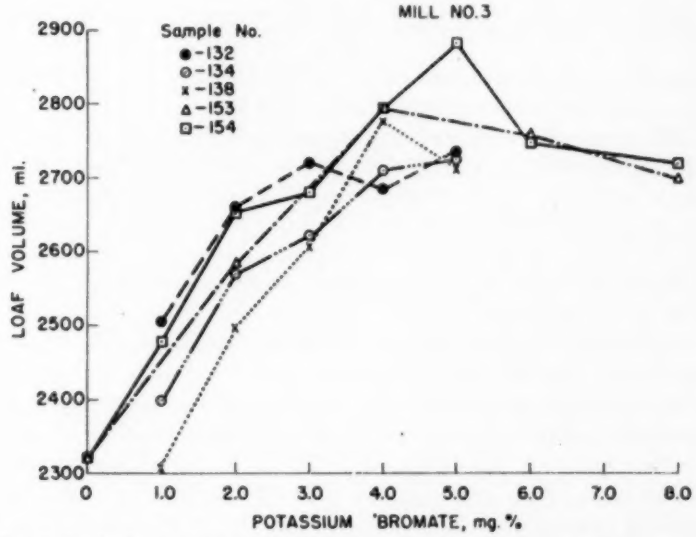

Fig. 3. Loaf-volume responses from addition of increments of potassium bromate to doughs containing 5% defatted soy flours obtained from Mill No. 3 over a 5-month period.

Loaf-volume response to potassium bromate is shown in Figs. 1–4. These graphs indicate a fairly good agreement in optimum bromate requirements as far as loaf volume alone is concerned. The optimum bromate requirements for the commercial soy flours show good agree-

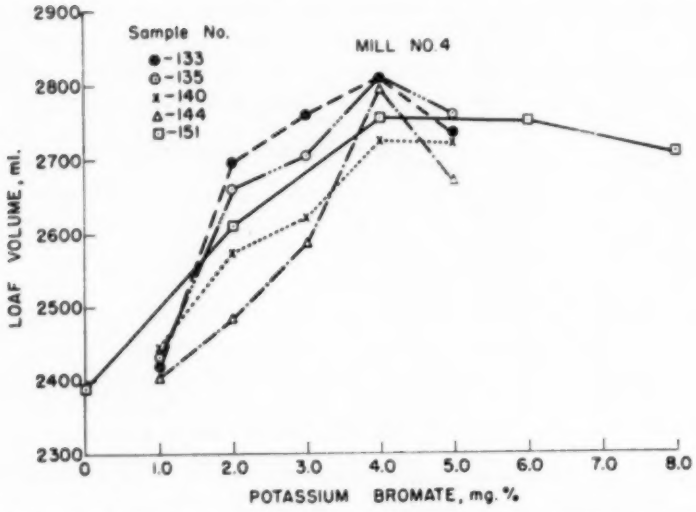

Fig. 4. Loaf-volume responses from addition of increments of potassium bromate to doughs containing 5% defatted soy flours obtained from Mill No. 4 over a 5-month period.

ment when all characteristics such as loaf volume, external loaf character, crumb grain, and texture are considered.

The values for optimum potassium bromate for all the soy flours tested are tabulated in Fig. 5. It must be remembered that these oxidation levels are applicable to this base flour when baked by the procedures previously outlined and under conditions existing in our laboratory during this study. It is probable that the oxidation levels employed here may not be applicable to other base flours and under other shop conditions.

Loaf-volume comparisons of the breads containing soy flour with bread made from the basic wheat flour plus 4% nonfat dry milk solids are shown in the bar graph in Fig. 5. These loaf volumes were not the maximum attainable, but rather the volumes obtained when each flour was baked at that oxidation level which represents the optimum over-all score, with internal and external characteristics and dough handling character considered as well as the loaf volume. Only one of the commercial soy flours did not yield a loaf volume in excess of that obtained with the addition of 4% nonfat dry milk solids to the basic wheat flour. Sixteen of the twenty soy flours produced bread with loaf volumes differing from that obtained with the basic loaf by amounts within those considered experimental error. The other four flours yielded loaf-volume differences slightly in excess of experimental

error and of these one yielded loaves which were larger than the basic loaf.

The addition of defatted soy flour had no effect upon proofing time. The average proofing time of all basic loaves was 59.8 minutes; that of all loaves containing soy flour, 59.6 minutes; and that of bread containing 4% nonfat dry milk solids, 55.6 minutes.

A factor which cannot be evaluated quantitatively but is most important, at least in the view of the baker, is texture, usually referred to in qualitative terms such as "silky" or "harsh." It is pertinent that, almost without exception, the addition of commercial soy flours to bread doughs yielded breads which had a harsh crumb as compared with the basic loaves.

Color of the crumb is another factor which is difficult to evaluate

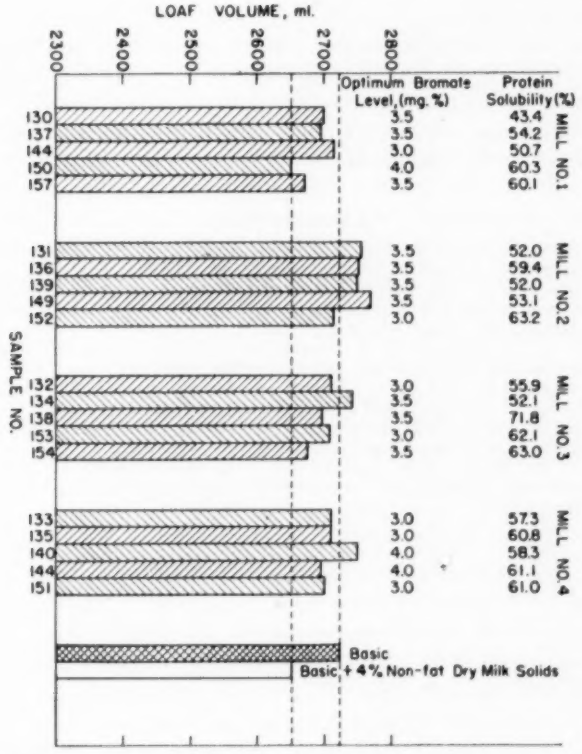

Fig. 5. Comparisons of the loaf volumes of breads containing 5% of each of 20 defatted soy flours baked at the indicated optimum potassium bromate level, with those of bread baked from the basic wheat flour alone and bread with 4% nonfat dry milk solids added. Also shown are the protein solubilities of the defatted soy flours.

quantitatively. Some of the commercial soy flours caused the bread crumb to appear whiter than the control. The use of a majority of the defatted soy flours, however, resulted in a crumb that had an off-white or gray cast that was more often dull than bright.

Determination of protein solubility (Fig. 5) showed that the commercial defatted soy flours had received a very minimum of heat treatment. Comparison of these data with the curves of Belter and Smith (1) indicates that the treatment was less than the equivalent of 5 minutes at atmospheric steam pressure.

There was no correlation between the baking performance of the soy flours and the values obtained for protein solubility.

Addition of the high-protein material, soy flour, to bread could greatly improve the nutritional adequacy of this basic foodstuff. Because of its high lysine content, soy flour may be the baker's most economical source of this amino acid for wheat protein supplementation.

When used with the proper amount of oxidation, defatted soy flour does not significantly impair loaf volume, crumb character, or dough-handling properties. Some soy flours improve crumb color, and it is doubtful that any color change caused by most soy flours would be noticeable to the consumer.

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A METHOD FOR THE QUANTITATIVE DETERMINATION OF ALBUMINS AND GLOBULINS IN WHEAT FLOUR[1]

JAMES W. PENCE, N. E. WEINSTEIN, AND DALE K. MECHAM

ABSTRACT

The albumin and globulin contents of wheat flour can be estimated quantitatively by a method developed from the characteristic tryptophan and amide nitrogen contents of the albumin, globulin, and gliadin proteins of flour. The proportions of the three types of protein contained in an extract of flour are calculated from the tryptophan and amide nitrogen values obtained for the mixture. The method provides a better estimate of the soluble proteins of flour than has heretofore been available because interference caused by varying amounts of gliadin in flour extracts has been largely eliminated and the proportions of albumins and globulins are determined by means of characteristic chemical properties. Results obtained with the method appear to be reliable within approximately $\pm 10\%$.

Most of the variation in the amount of protein extracted from a flour by solutions of different salts appears to be due to variation in the solubility of gliadin in such solutions, although variation in the solubility of albumin and globulin components was also observed. Sodium chloride appeared to be the best of those salts examined for the simultaneous extraction of the largest amounts of both the albumins and globulins from flour.

The soluble proteins of wheat flour, particularly the albumins, have been found to be necessary for the adequate performance of reconstituted bread doughs, at least under the experimental conditions used by Pence, Elder, and Mecham (10). As the soluble proteins appear to consist of a complex mixture of albumins and globulins (8, 9), satisfactory methods for quantitative determination of each type of these proteins are essential for study of relations between the protein composition of flours and variations in baking performance among flours.

Early methods for determination of the soluble protein contents of flour (1, 7, 11) usually relied on extraction with dilute salt solutions and showed little concern for the identity of substances extracted, although the presence of significant amounts of gliadin was noted by several investigators (2, 5, 7). Separations by dialysis or heat coagulation were only occasionally used in attempts to distinguish between albumins and globulins (2, 13). The amount of protein extracted from flour with dilute salt solutions was shown by Gortner, Hoffman, and Sinclair (4) to depend greatly on the kind and concentration of the salt used. These investigators, however, gave no information as to the identity of the proteins extracted with the various salts.

[1] Manuscript received August 17, 1953. Contribution from Western Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture, Albany, California.

Recent study of the soluble proteins of flour in this laboratory (9) has provided new criteria for identification of the albumin and globulin proteins, based on characteristic chemical and physical properties. The present paper describes a method of analysis derived from the chemical properties of these proteins which can be used to study the composition of extracts of flour made with different salt solutions. The quantities of albumin, globulin, and gliadin contained by the mixture of these proteins are calculated from the tryptophan and amide nitrogen contents of such extracts.

Materials and Methods

Soluble proteins were extracted from flour by slowly sifting 40 g. of flour into 350 ml. of 0.5 *M* sodium chloride (buffered at pH 6.8 with 0.1% phosphates) contained in an electric blender running at a slow speed so as to suspend the flour smoothly and with a minimum of foam formation. The suspension was then poured into two 250-ml. centrifuge bottles and shaken gently by mechanical means for 30 to 40 minutes. The suspension was centrifuged and the supernatant decanted. The flour solids were returned to the blender twice more and stirred for 5 to 10 minutes with 300 to 350 ml. of fresh extractant. The supernatants were combined, and aliquots (50 to 100 ml.) of the final volume were taken for determination of the total and nonprotein nitrogen extracted from the flour. The remainder of the combined supernatants was dialyzed overnight against running tap water followed by two changes of distilled water to remove salts and dialyzable nonprotein nitrogenous components. The dialyzed extract was concentrated under vacuum and dried by lyophilization. Yields were usually between 1.4 and 1.8 g. of dry material containing 6 to 9% of nitrogen.

Tryptophan was determined by the colorimetric method of Spies and Chambers (12), and amide nitrogen as described by Mecham and Olcott (6). Nonprotein nitrogen was determined as the nitrogen remaining in solution after tungstic acid treatment by the method used by Blish and Sandstedt (3).

The flour samples used included several straight-grade flours, experimentally milled from pure-variety lots of wheat. In addition, an unbleached, commercial cracker flour and bleached and unbleached samples of a commercial, second-middlings flour were used.

Results and Discussion

Results reported recently (9) indicate that flour albumins possess

a tryptophan content that is characteristically high for this group of soluble proteins, whereas globulins were found to have a much lower tryptophan content—similar to that of gliadin, which appears to be the main contaminant of preparations of soluble flour proteins. Gliadin is characterized by an amide nitrogen content much higher than those of the soluble proteins. Because only these three types of protein appear to be present to a significant extent in dilute salt extracts of flour, their compositional characteristics can be used to calculate the approximate proportions of each protein contained in the extracts.

Calculation of the Protein Composition of Flour Extracts.

$$\text{Let} \quad x + y + z = 1, \qquad \text{(a)}$$
$$Ax + Cy + Ez = M, \text{ and} \qquad \text{(b)}$$
$$Bx + Dy + Fz = N \qquad \text{(c)}$$

The letters x, y, and z represent the fraction of total protein nitrogen of the extract contained by the albumin, globulin, and gliadin components, and M and N signify the amide and tryptophan nitrogen contents of the extracted protein material. A and B, C and D, and E and F represent the amide and tryptophan nitrogen contents, respectively, of purified albumins, globulins, and gliadin as shown in the following table:

Protein	*% Amide N of Total N*	*% Tryptophan N of Total N*
Albumins	8.4	2.57
Globulins	6.0	0.73
Gliadin	25.0	0.65

By substitution of these values in the above equations and solving for x, y, and z, the following expressions are obtained:

$$x = \quad 0.0023\,M + 0.540\;N - 0.408 \qquad \text{(d)}$$
$$y = -0.0547\,M - 0.472\;N + 1.672 \qquad \text{(e)}$$
$$z = \quad 0.0524\,M - 0.0683\,N - 0.264 \qquad \text{(f)}$$

The relative proportions of albumins, globulins, and gliadin in the mixture are thus calculated, and by multiplying x and y with the quantity of extracted protein, the albumin and globulin contents of the flour are obtained.

The reliability of the calculations for the proportions of each type of protein in an unknown mixture depends greatly on the accuracy with which characteristic amide and trypophan contents can be assigned to each class of the proteins. The values used for these constants were obtained from the results given in Tables I, II, and III. The small variations in chemical composition of the albumin and

TABLE I

TRYPTOPHAN AND AMIDE NITROGEN CONTENTS OF ALBUMIN PREPARATIONS FROM DIFFERENT FLOURS

Flour	Wheat Type	Tryptophan N[1]	Amide N[1]
		%	%
Commercial, unbleached	White	2.60	8.3
Turkey	HRW	2.61	8.1
Pentad	Durum	2.45	7.7
Chiefkan	HRW	2.50	8.3
Comanche	HRW	2.60	8.1
Rex	White	2.79	8.4
Red Chief	HRW	2.50	8.9
Thatcher	HRS	2.51	8.9
Baart	White	2.55	8.9
Mean		2.57	8.4
Coefficient of Variation		4.1	5.6

[1] Expressed as % of total nitrogen.

TABLE II

TRYPTOPHAN AND AMIDE NITROGEN CONTENTS OF DIFFERENT GLOBULIN PREPARATIONS FROM WHEAT FLOUR AND GERM

Source	Estimated Ratios of Separate Components (α:γ:δ)[1]	Tryptophan N[2]	Amide N[2]
		%	%
Germ	0:74:20	0.81	6.0
Germ	20:68:11	0.65	6.0
Flour	44:46:7	0.73	6.1
Germ	27:52:16	0.80	5.9
Germ	10:70:17	0.71	6.0
Germ	13:70:11	0.69	6.1
Mean		0.73	6.0
Coefficient of Variation		8.6	1.3

[1] Estimated by measurement of areas under peaks on sedimentation diagrams (10).
[2] Expressed as % of total nitrogen.

globulin preparations listed in Tables I and II do not appear to have any systematic relation with variations in component composition as found by physical means (9). The so-called gliadin preparations listed in Table III were isolated from the indicated extracts of flour and are believed to represent the major protein contamination of albumins and globulins contained in such extracts. The amide nitrogen content of this gliadinlike material is lower than the 25.5 to 26.5% of total nitrogen usually reported for gliadin (1). Similarly,

TABLE III

TRYPTOPHAN AND AMIDE NITROGEN CONTENTS OF GLIADINLIKE FRACTIONS ISOLATED FROM WATER OR DILUTE-SALT EXTRACTS OF FLOUR[1]

Solvent	Tryptophan N[2]	Amide N[2]
	%	%
Distilled water	0.69	25.1
0.1% Phosphate buffer, pH 6.8	0.56	25.8
0.1% Phosphate buffer, pH 6.8	0.63	24.8
0.5 *M* Sodium bromide	0.70	24.1
Mean	0.65	25.0
Coefficient of Variation	10.0	2.8

[1] Precipitated from extract with 0.4 M ammonium sulfate; precipitate taken up in 60% alcohol; heated to boiling for 30 min.; dialyzed against 60% alcohol; and the soluble fraction recovered by centrifugation and lyophilization.
[2] Expressed as % of total nitrogen.

the tryptophan nitrogen content of this gliadinlike material is lower than the average of 0.73% of total nitrogen found in this laboratory for samples of gliadin prepared from gluten by customary procedures and having amide nitrogen contents between 26.0 and 26.5% of total nitrogen. The lower-than-normal tryptophan values for the gliadinlike material indicate that the low amide nitrogen values also obtained are not the result of admixtures of gliadin with albumins or globulins, provided the average tryptophan content of the latter is correct as shown in Table II. Neither is it likely that an unknown protein of low tryptophan content is mixed in small amounts with gliadin, because, as larger amounts of gliadin are extracted (with sodium bromide, for instance), the effect of such a protein should diminish. The results in Table III do not support such an interpretation.

The coefficients of variation for the amide and tryptophan contents of the three classes of protein are all 10% or less of the mean values, a result which is compatible with the over-all experimental error discussed in a later section of this paper.

Extraction of Soluble Proteins by Various Salts

Because Gortner, Hoffman, and Sinclair (4) showed that the amount of nitrogen extracted from a flour by salt solutions depended greatly upon the kind and concentration of salt used, the composition of protein material extracted from flour by various salts was investigated. Samples of a flour were extracted, in duplicate, with 0.5 *M* solutions of the salts listed in Table IV, and the composition of the extracted material was determined by the method described above.

TABLE IV

ALBUMIN AND GLOBULIN CONTENTS OF A FLOUR (RED CHIEF) AS DETERMINED BY EXTRACTION WITH 0.5 *M* SOLUTIONS OF VARIOUS SALTS[1]

Salt	Protein N Extracted From Flour	Protein N of Flour Expressed as:		
		Albumin	Globulin	Gliadin[2]
	%	%	%	%
LiCl	22.9	8.3	6.0	8.7
NaF	13.5	6.0	4.7	1.3
NaCl	21.4	7.6	7.6	6.2
NaBr	32.7	9.6	6.5	16.7
$MgCl_2$	29.9	9.2	7.1	15.5
$CaCl_2$	27.8	8.2	6.6	14.8
$MgSO_4$	16.9	7.2	8.2	1.5
K_2SO_4	17.0	6.7	8.0	2.3
Na_2BO_3	22.6	7.7	3.7	11.2

[1] Solutions adjusted to pH between 6 and 7 before use.
[2] Gliadin contents of the extracts are expressed here as % of protein of the flour for convenience of comparison only.

As expected, a large variation was found in the total amount of protein nitrogen extracted by the various salts. With one exception (Ca^{++} *vs.* Mg^{++}), the results obtained agree with those found by Gortner *et al.* (4). A large variation was also found in the amounts of the three types of protein contained in the extracted material, particularly with respect to gliadin. Amounts of the latter show a pronounced tendency to vary directly with the quantity of total protein extracted, whereas the amounts of the other two proteins show more of a tendency to approach an upper limit, unrelated to the amount of total protein extracted. Apparently, a much greater part of the variation observed in the quantity of protein extracted from flour by solutions of different salts is due to variation in the solubility of gliadin than of albumins and globulins in the solutions.

Of the three salts which extracted the largest amounts of globulin (sodium chloride, magnesium sulfate, and potassium sulfate), only sodium chloride extracted an amount of albumin which approached the apparent maximum extractable. Sodium bromide and magnesium chloride extracted the two largest amounts of albumin, but also the largest amounts of gliadin and less than maximum amounts of globulin.

On the basis of these results, sodium chloride appears to be the best single salt to use for the simultaneous extraction of the largest amounts of both albumins and globulins. This conclusion is supported by the results shown in Table V, which compares the amounts of the proteins extracted by 0.5 *M* solutions of sodium chloride and sodium

TABLE V

COMPARISON OF AMOUNTS OF ALBUMINS AND GLOBULINS EXTRACTED FROM DIFFERENT FLOURS WITH 0.5 *M* SOLUTIONS OF SODIUM CHLORIDE AND SODIUM BROMIDE

Flour	Albumins[1]		Globulins[1]	
	NaCl	NaBr	NaCl	NaBr
	%	%	%	%
Thatcher	7.8	5.8	5.8	7.8
Tenmarq	7.5	7.0	9.4	9.7
Purkof	10.7	9.7	7.8	9.9
Red Chief	7.6	9.6	7.6	6.5

[1] Expressed as % of total flour protein.

bromide from several different flours. With the exception of the Red Chief flour (used in Table IV), sodium chloride extracted a greater amount of the albumins than did sodium bromide. On the other hand, sodium bromide definitely extracted more of the globulins from the Purkof and Thatcher flours than did sodium chloride.

The extent to which solubility characteristics of individual albumin or globulin components contribute to these variations cannot be stated, as yet, but it has been observed that up to 25% of albumins separated from a 0.1% phosphate extract of flour are precipitated by added potassium sulfate (0.5 *M* final concentration). Krejci and Svedberg (5) similarly observed that the molecular weights of proteins extracted from flour with 0.5 *M* solutions of alkali halides increased when potassium bromide was used in place of sodium chloride.

Until adequate information regarding the relationships among individual protein components and their solubilities in different salt solutions becomes available, an unknown degree of uncertainty must accompany application of the proposed method to flours in general.

Reproducibility of Results

The nature and number of manipulations necessary for preparation of the flour extracts in the present study introduce many sources of experimental error. An estimate of the size of this error and the reproducibility of results obtained with the proposed method was obtained from the typical values shown in Table VI. Albumin and globulin determinations were run in quadruplicate on a sample of commercial, second-middlings flour which had been treated with chlorine dioxide. As no significant difference was found between values for this flour and its unbleached control run in triplicate, seven

TABLE VI

REPRODUCIBILITY OF ANALYTICAL DETERMINATIONS OF ALBUMIN AND GLOBULIN CONTENTS OF A FLOUR

Run No.	Albumin[1]	Globulin[1]
	%	%
1	8.3	6.6
2	8.1	5.7
3	8.3	5.5
4	7.9	6.0
5	9.2	4.8
6	8.1	5.8
7	8.9	5.0
Mean	8.4	5.6
Standard Deviation	0.5	0.6
Coefficient of Variation	6.0	11.7
Minimum Significant Difference[2]	0.6	0.7

[1] Expressed as % of total flour protein.
[2] At 5% point.

replicates were therefore available for statistical evaluation. The minimum significant differences (at the 5% point) between albumin and globulin contents obtained for the separate runs were thus found to be 0.6 and 0.7% of the total flour protein, respectively. The corresponding coefficients of variation were 6.0% and 11.7% of the means.

Despite the limitations and the empirical and somewhat arbitrary nature of the proposed method, it appears to offer a better estimate of the soluble protein contents of flour than has heretofore been available. The disturbing effect of varying amounts of gliadin present in flour extracts can largely be avoided, and the proportions of albumin and globulin obtained are based on characteristic chemical properties of the respective proteins. It is anticipated that the empirical nature of the method can be reduced substantially as more detailed knowledge is gained regarding the chemical and solubility properties of individual components of the soluble proteins of flours.

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A ROUTINE METHOD FOR ESTIMATING THE STARCH CONTENT OF WHEAT BY-PRODUCTS[1]

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ABSTRACT

The starch content of mill by-products may be conveniently determined by removing the soluble carbohydrates with 2% hydrochloric acid solution, hydrolyzing the residue by boiling with hydrochloric acid, and determining the reducing power of the digest before and after yeast fermentation. The difference represents glucose which arose from the hydrolysis of starch. Assuming that wheat endosperm contains 81% starch, the flour content of the mill streams can be readily calculated. Samples of pollard from Irish mills contained from 13.4 to 21.6% starch; the values for bran ranged between 10.5 and 13.1%. Two sets of samples from American mills gave the following starch contents: sharps, 26.1, 27.9%; red dog, 36.9, 46.6%; bran, 12.9, 18.2%.

The residual sugars after fermentation represent pentoses from the insoluble pentosans.

In the Republic of Ireland, flour extraction is specified at 85%[3] of the weight of the clean wheat by government order. Commercial flours vary appreciably in color and ash content, indicating that different mills achieve varying degrees of success in separating the endosperm from the bran and germ. A knowledge of the flour content of the by-products, bran and pollard (shorts), based on their starch content, would be of value.

For mill control, it is necessary to use a simple and rapid method. Preliminary trials showed that published methods were unsuitable for various reasons. The Rask procedure (1) involves tedious preliminary

[1] Manuscript received May 26, 1953.
[2] Dublin Port Milling Co., Ltd., Dublin, Ireland.
[3] Since this paper was written the extraction has been reduced to 80%.

extraction, and its modification according to Kent-Jones and Herd (4) requires the removal of protein. In the method of Chinoy, Edwards, and Nanji, described by Kent-Jones and Amos (5), the extraction of the starch is difficult and the final washing of the starch iodide with alcohol results in an indeterminate loss. The A.O.A.C. method, which involves solubilization of the starch with amylase followed by acid hydrolysis, requires the use of a high-amylase malt and involves tedious filtrations.

The modified hydrolysis procedure described in this paper was developed for the routine determination of starch and insoluble pentosans in wheat by-products.

Routine Procedure for the Determination of Starch and Insoluble Pentosans in Wheat By-Products. A 1.0-g. sample in each of two 50-ml. centrifuge tubes is treated with dilute hydrochloric acid solution (2% hydrochloric acid by volume plus 0.5 ml. Teepol[4] per l.) with occasional stirring for 10 minutes. The suspension in each tube is centrifuged at about 2500 r.p.m. for 7 minutes; the supernatant is removed by suction tubes and filtered through No. 41 Whatman paper. After two further extractions in this manner, the residues in both centrifuge tubes and on the filter paper are transferred to a 500-ml. flask with 180 ml. of water. Twenty ml. hydrochloric acid solution (sp. gr. 1.125) and a few pieces of pumice stone are added and the suspension is boiled gently for 2.5 hours. The solution is transferred to a 250-ml. volumetric flask, made up to volume at 20°C., and filtered through No. 40 Whatman paper. The acidity in a 15-ml. aliquot of the filtrate is neutralized with solid sodium carbonate, using a few drops of methyl orange solution as indicator, and the reducing power determined by the Schoorl method (3). A 150-ml. aliquot of the filtrate in a 400-ml. beaker is adjusted to pH 6.0, with 30% sodium hydroxide solution and finally with 0.1 *N* sodium hydroxide solution. The temperature is adjusted to 30°C., 1.0 g. of washed baker's yeast added, and the mixture fermented for 16 hours at 30°C. After cooling to 20°C., the volume is made up to 250 ml., the suspension centrifuged, and the reducing power of 25 ml. of the supernatant determined by the Schoorl method.

The aliquots for each reduction are equivalent to 120 mg. of the original sample. The thiosulfate equivalent to the glucose formed by acid hydrolysis = (B − X) − (B − Y) = Y − X, where B = the thiosulfate blank, and X and Y are the volumes of thiosulfate used in titrating the solution before and after fermentation, respectively.

[4] A sulfonated hydrocarbon, manufactured by Shell, Ltd., London, England.

Percentage starch $= (0.93G \times 100)/120 = 0.775G$ where G = mg. glucose corresponding to the thiosulfate titer (Y − X).

The factor 0.93 for conversion of glucose to starch was found by determinations on pure wheat starch and is in agreement with the findings of other workers (2).

The approximate percentage of endosperm or flour $= (0.93G \times 100 \times 100)/(120 \times 69.3) = 1.12$ G, assuming that wheat endosperm contains 81% starch on a dry basis (Shetlar *et al.*, 6), and therefore 69.3% starch at 14.5% moisture.

The reduction after fermentation is due to the pentoses formed from the water-insoluble pentosans by acid hydrolysis. Assuming that the mixture of xylose and arabinose has the same reducing power as glucose, the approximate percentage of insoluble pentosans = (mg. pentose [from glucose table] × 132 × 100)/(150 × 200) = mg. pentose × 0.73. The factor 132/150 provides for the conversion of pentose to pentosan.

The extraction with dilute hydrochloric acid removes dextrins and water-soluble pentosans as well as sugars. The wetting agent (Teepol) was used to wet the small starch granules, which otherwise would float on the surface of the solution in the centrifuge tube. Determinations of reducing sugar were made on the respective filtrates from four successive extractions on three samples of by-products with the following results:

Extraction No.	*0.1 N Thiosulfate Solution*[1]		
	Pollard No. 1	*Pollard No. 2*	*Bran*
	ml.	*ml.*	*ml.*
1	8.7	8.9	8.0
2	1.2	1.4	1.5
3	0.0	0.1	0.2
4	0.1	0.0	0.1

[1] The values represent the thiosulfate corresponding to the copper reduced by an aliquot of the filtrate equivalent to 0.6 g. of sample.

No comparisons were made with other methods, but recovery tests were carried out by adding 0.1 g. of wheat starch (containing 85.2% starch, as estimated by difference after deducting the moisture, protein, fat by acid hydrolysis, and ash contents) to 2.0 g. of bran or pollard and determining the total starch before and after this addition. The results of replicate tests are given in Table I.

These recovery experiments indicate that the method is accurate within about ±2%.

TABLE I

RESULTS OF STARCH RECOVERY EXPERIMENTS WITH POLLARD AND BRAN

Replicate No.	Starch Content		Starch Recovered	Percentage of Recovery
	Untreated	With Added Starch		
	mg.	*mg.*	*mg.*	
Pollard				
1	432.4	520.8	88.4	103.7
2	342.2	426.4	84.2	99.1
3	410.3	493.8	83.5	98.2
Bran				
1	206.0	289.2	83.2	97.9
2	262.0	348.8	86.8	102.1
3	252.3	337.7	85.4	100.2

The reducing sugars which remain after yeast fermentation represent the pentoses derived by acid hydrolysis of the pentosans which are not dissolved and removed by the extractions with 2% hydrochloric acid solution. Tests with pure samples of L-arabinose and D-xylose showed that they were not fermented by the yeast used in these studies.

Results

The method has been applied to the routine analysis of mill by-products for which mill yield figures were available. From these yields and the estimated flour content of the samples, the amount of flour lost in the mill offals may be calculated. Typical data are given in Table II.

Discussion

The data of Table II are self-explanatory; a few comments would, however, be apposite. Mill 4 would be rated the most efficient, showing the lowest flour loss coupled with the lowest total by-product yield. Mill 5 is the least efficient. The data for mills 2 and 3 show them to be about average in this respect. The high total by-product yield of mill 1, together with the average flour loss figure, would indicate a good standard of milling technic; the indications here are that the wheat used contained a high proportion of low flour-yielding wheats, due to shriveled or frosted grains.

The spread of flour loss figures is greater in the pollard samples than in the bran samples; this is as would be expected. The general indications are that if the pollard flour content rises above 25% at 85% flour extraction, then undue flour loss is taking place.

TABLE II

THE STARCH, FLOUR, AND INSOLUBLE PENTOSAN CONTENT OF WHEAT MILL BY-PRODUCTS

Mill No.	Material	Mill Yield[1]	Starch Content	Estimated Flour Content	Flour in Product	Total Flour Lost	Insoluble Pentosans
		%	%	%	%	%	%
				Irish Sources			
1	Pollard	10.1	14.3	20.6	2.1	3.1	22.3
	Bran	6.8	10.5	15.1	1.0		25.8
2	Pollard	9.2	16.1	23.1	2.1	3.1	21.2
	Bran	5.9	11.5	16.6	1.0		25.2
3	Pollard	10.7	16.2	23.4	2.5	3.4	21.8
	Bran	5.0	11.8	17.0	0.9		24.5
4	Pollard	7.9	13.4	19.3	1.5	2.8	23.1
	Bran	6.9	13.1	18.9	1.3		23.4
5	Pollard	10.0	21.6	31.2	3.1	4.2	18.2
	Bran	5.9	12.6	18.2	1.1		24.7
				American Sources			
6	Red Dog	2.7	46.6	67.2	1.8	8.6	6.4
	Sharps	11.9	27.9	40.3	4.8		12.9
	Bran	11.0	12.9	18.6	2.0		22.0
7	Red Dog	3.6	36.9	53.3	1.9	8.0	9.2
	Sharps	11.6	26.1	37.7	4.4		15.0
	Bran	6.5	18.2	26.2	1.7		18.4

[1] Percentages are expressed on the basis of clean wheat going to the mill.

The great difference in flour losses between Irish and American samples may be reasonably regarded as being largely due to the difference in flour extraction required in the two countries; they could not fairly be viewed as an indication of the relative milling efficiency. It would indeed be an almost insoluble problem to rate, in order of efficiency, two mills operating at 85% and 72% flour extraction respectively. It is, however, only fair to say that almost certainly the

Irish mills are the more efficient in flour extraction, for the very obvious reason that much greater effort over a long period of time has been expended on every device to increase the extraction.

Acknowledgment

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THE MICROSCOPIC STRUCTURE OF BREAD AND DOUGH[1, 2]

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ABSTRACT

A method for obtaining photomicrographs showing the microscopic structure of doughs and of bread is described. The dough or bread is embedded in plastic and sectioned by grinding. Only the surface exposed by grinding is stained, and reflected light is used for taking the pictures; hence the need for thin sections is avoided. Photomicrographs of cross sections of gas cell walls of dough and bread indicate that there is no segregation of starch or of protein to isolated positions in the films. In general, starch granules are separated from each other by the protein, which is the continuous phase of the film. Granules are oriented by the pull of the expanding gas bubble during fermentation. After gelatinization during baking, further expansion of gas bubbles causes a pronounced distortion of the then flexible granules. However, under normal baking conditions, granules do not disintegrate but retain their identity.

A true picture of the structure of the films which form the walls of gas cells of bread would be a valuable contribution to a number of studies involving baking, such as the role of starch in baking and in bread staling and the effects on bread structure of various treatments with enzymes, oxidizing agents, and reducing agents. As stated by Baker (1), "The structure and properties of the walls must of necessity determine the character of the dough." Some recent theories concerning the relation of starch and its modifications to the structure of doughs and bread (4) indicate a need for knowledge of the structure.

Studies of the structure of bread, by a number of investigators, were adequately reviewed by Burhans and Clapp (2), who pointed out the difficulties involved in making cross sections of films and outlined precautions that are necessary to avoid artifacts.

Attempts to section and photograph bread films by the usual botanical technics have not been satisfactory (3, 5). The wetting and drying of the films occasioned by these methods cause distortions which may be misinterpreted. Photographing by light transmitted through the sample makes thin films necessary. The Burhans and Clapp sections were cut from material frozen with carbon dioxide,

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The subject matter of this paper has been undertaken in cooperation with the Quartermaster Food & Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.
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with fixation and staining with osmic acid vapor, dehydration with calcium oxide, embedding with paraffin, and cutting with a sliding microtome. This procedure gave satisfactory sections. Baker (1) noted that a bread film was not colored by iodine except where the film had been cut or ruptured—e.g., a scratch on the surface would be colored. He concluded that the films of bread had walls of impervious glutenous material, and that the starch granules were completely covered by a protein layer and were more or less segregated to the interior of the film. Baker did not confirm this theory by making cross sections of the walls.

Studies in this laboratory concerning the relation of starch to baking properties and the role of starch to bread staling required a knowledge of the microscopic structure of bread. Since botanical methods had been shown to be unsatisfactory, geological methods which were in use in a study of wheat-kernel structure were applied to this study also.

Methods

In order to avoid artifacts, certain precautions in the study of bread structure must be observed. Bread crumb will take up about five times its weight of water; this is largely due to absorption by the starch. Accordingly, a bread film as observed in water or stained by a water solution of stain would present a distorted picture. Allowing the films to dry, using dehydrating agents in the preparation of the film for sectioning, and staining with stains in dehydrating solvents are equally undesirable, since the shrinkage of the starch and protein causes breaks between starch granules and protein which may be misinterpreted (5).

Crude sections of bread crumb, cut by hand with a razor blade, were immersed in liquid plastic containing a hardening catalyst.[4] Infiltration of plastic into the crumb was facilitated by removal of gas by reduced pressure. The plastic-embedded bread crumb was further sectioned by grinding (in a manner similar to methods used for sectioning geological specimens). The surface exposed by grinding was stained with a 1% solution of Cotton C_4 Blue[5] in 80% glycerine. Iodine, acid or basic fuchsin, or other stains in 80% glycerine may be used. This concentration of glycerine was chosen because it caused little shrinkage or swelling of the starch or protein. The Cotton C_4 Blue dyes the protein blue, leaving the starch white. Fortunately the

[4] Plasticast C, Plasticast Drier, and Special Plasticast Catalyst, Plasticast Company, Palo Alto, California.
[5] Eimer and Amend, New York, N. Y.

stains penetrate only slowly into the protein matrix of the film; thus the staining may be limited to the immediate surface and the confusion due to viewing stained protein (or starch) in the background as well as at the surface is avoided. When the desired intensity and depth of color were obtained, the excess staining liquid was removed and the surface was covered with mineral oil for study under the microscope.

Light reflected from the surface of the sample rather than light transmitted through the sample was used for microscopic observations and photomicrographs. Reflected light permits the use of thick sections with only the exposed surface stained to show the structure.

Cross sections of dough films are more difficult to obtain than those of bread films. Retention of shape and structure of a dough film is dependent on retention of gas pressure in the bubble; the film does not stand up without support. Preparation of dough sections was similar to that of cross sections of bread films, except that small pieces of dough, after 2 hours' fermentation, were immersed in liquid plastic and then subjected to reduced pressure. The resulting expansion and rupture of gas cells allowed infiltration of plastic into the cell structure. The infiltered plastic gave sufficient rigidity to the cell walls so that crude sections could be cut with a razor blade. These sections were again immersed in plastic and, after hardening, were ground to expose the edges of the dough films.

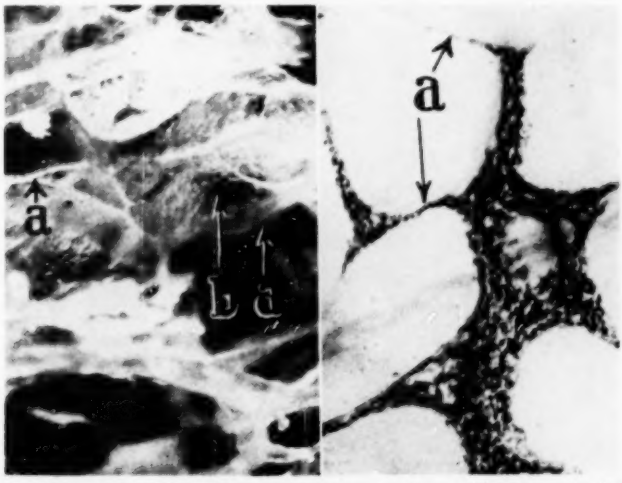

Fig. 1. Comparison of the microscopic appearance of normal bread crumb (left) to the edgewise view as obtained in embedded stained crumb (right) (×30): a, cross sections of films; b, surface views of films.

Results and Discussion

For general orientation, Fig. 1 shows a low-power over-all microscopic view of a slice of bread crumb before embedding or staining (left) as compared to an embedded, ground, and stained crumb (right). Embedding eliminates confusing background and limits the view and staining to the immediate exposed cut surface. The section cuts through the gas cell walls at various angles, thus showing films cut parallel (tangent) to the surface *(b)* as well as films cut across the surface *(a)*.

Figure 2 shows edgewise views of dough film structure before baking and, accordingly, before the starch has been gelatinized and thus made flexible. Even in the dough the starch granules show orientation in reference to the film surface, but there is no evident segregation of granules or protein. For contrast, Fig. 3 shows the disorderly or nonoriented arrangement of granules in a solid piece of dough (a newly mixed dough with no gas cells).

Figure 2, *c* and *d*, shows the edges of thin dough films. The strength of such films would seem to be due to a strong adherence of protein to starch; otherwise starch would be forced out of the films and on

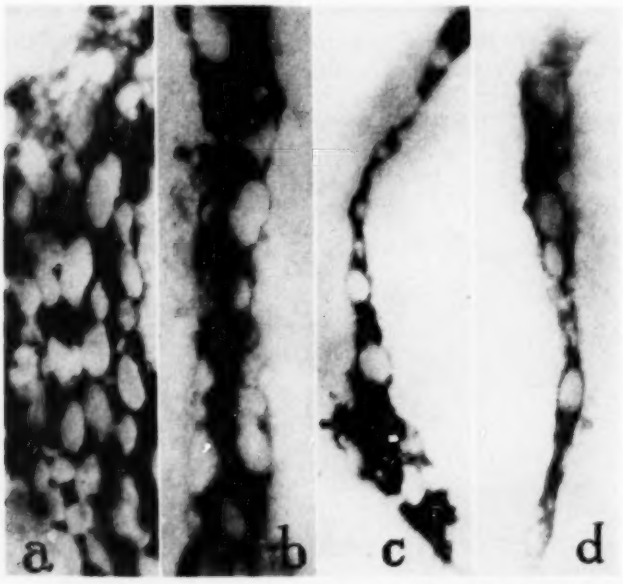

Fig. 2. Edgewise views of dough films showing orientation and distribution of granules; Cotton C. Blue stain (×400): a, thick portion of film at junction of gas cells; b, c, d, cross sections of thin films.

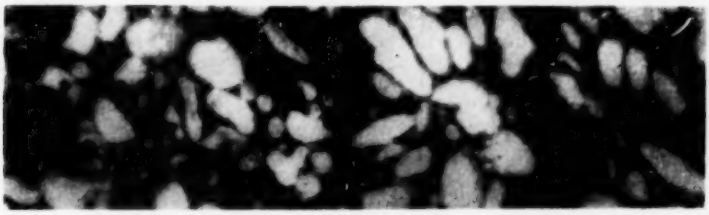

Fig. 3. Nonoriented granule arrangement in a freshly mixed dough with no gas cells (×400).

gelatinization these starch granules would probably become the weak points in the films. Granules on the surface of dough films stain readily with n/250 iodine in water solution in contrast to granules of bread films (1).

During baking, as gas cells expand with increasing temperature, the pull on granules in the gas cell film is parallel to the film surface. Before gelatinization, this tends to orient the granules (as indicated in Fig. 2). However, as granules are gelatinized and thus softened and made flexible, they become greatly dilated and more completely oriented, as shown in Fig. 4. This higher magnification (×200) illustrates the orientation and elongation of starch granules caused by cell expansion. The appearance of cross sections of film is partially determined by the angle at which they are cut. This is illustrated in Fig. 4: Film *a* was not necessarily a thick film but may possibly have

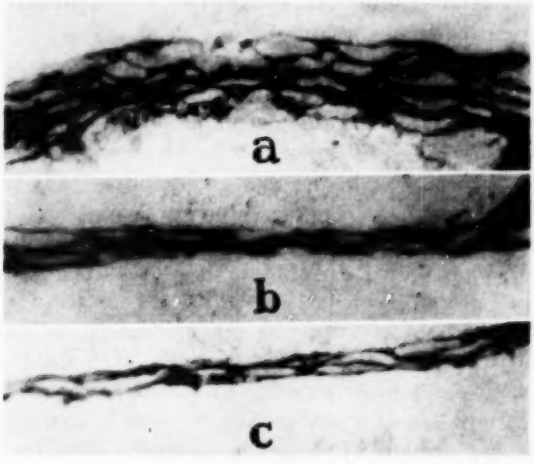

Fig. 4. Orientation and elongation of starch granules in cross sections of bread films (×200).

been a thin one cut at an acute angle to the surface. A perpendicular surface view of a film does not show orientation (1, 2, 3).

Figure 5 is a higher magnification (×400) of cross sections of relatively thin bread films, showing an unequal distribution of large and small granules and of protein throughout the film, but no actual segregation to definite positions in the film.

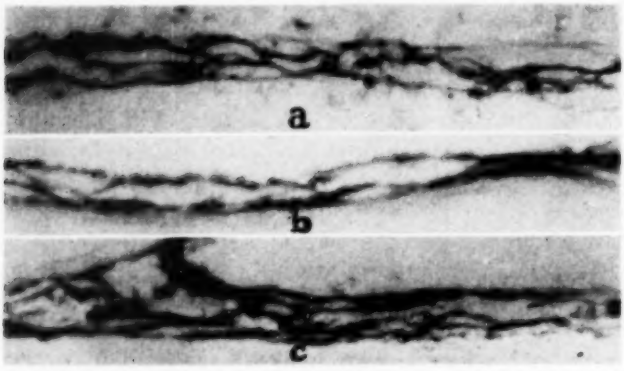

Fig. 5. Higher magnification of bread films to show unequal distribution of large and small granules and of protein (×400): b and c, films indicating coalescence of starch granules—baked from dough containing a bacterial enzyme preparation.

Figure 5, *b*, shows a thin film of bread. When a film has reached maximum expansion and is perhaps little thicker than an individual granule, the gelatinized granules probably are weak points in the structure and cause rupture of the film.

In Figure 5, *b* and *c* indicate possible coalescence of starch granules under certain conditions. These are films from a loaf baked from dough containing a bacterial enzyme preparation.

These photomicrographs indicate that, under the usual conditions of baking, individual starch granules are deformed but retain their identity, even though gelatinized. The granules do not disintegrate, probably because their swelling is limited by the deficiency of water (40% to 45% moisture). Each granule appears to be separated from the others by a protein layer, the protein being the continuous phase in the film. Baker's observation (1) that the starch in an unbroken film does not stain indicates that all granules, even those at the surface, are covered by a protein layer. This may further indicate that under these conditions of water deficiency there is a strong bond between starch and protein. However, in an excess of water the starch is easily washed from the protein. In general the granules do not touch

each other. They are not segregated to a particular position but are distributed throughout the film – how well distributed could be one of the factors determining the strength of the film.

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A NOTE ON STARCH OF HIGH AMYLOSE CONTENT FROM CORN WITH HIGH STARCH CONTENT[1]

W. L. Deatherage,[2] M. M. MacMasters,[2]
M. L. Vineyard,[3] and Robert P. Bear[3]

Native starches are known to vary considerably in the ratio of amylose to amylopectin which they contain. The waxy or glutinous cereal starches contain little or no amylose (1, 7, 10), while starch from wrinkled-seeded, garden-type peas contains from about 60 to 70% amylose (5, 8). With two exceptions, all other reports of the amylose content of various starches indicate values between these two extremes. The first exception is a report that Steadfast variety peas contain starch which, as determined by a combination of experimental methods, is 98% amylose (9). However, studies made at the Northern Regional Research Laboratory of starch from a sample of Steadfast variety peas obtained from England for the purpose indicated that the amylose content of the starch was 65% (unpublished data) as determined by potentiometric titration with iodine (5). The second exception is a recent publication in which a corn with starch containing 77% amylose was reported (3).

It has been suggested that a starch of high amylose content could be used, without fractionation, for the preparation of films similar to those that have been made experimentally from amylose obtained by the fractionation of starch (11). Because of existing industrial facilities in the United States, the most desirable source of high-amylose starch would be corn. The maximum amylose content of cornstarch reported up to this time, however, is 77% (3), and cornstarch of high amylose content has been found only in "sugary" corn with a low starch content (2, 3, 4, 6).

It is now possible to report that corn with a high starch content has been bred, which contains starch of 55 to 60% amylose content. The development of the corn by the Bear Hybrid Corn Company will be reported elsewhere.

A series of strains of corn of a new endosperm type has been produced, in which the amylose content of the starch varies with the genetic composition of the corn. The amylose content of the starch

[1] Manuscript received June 22, 1953.
[2] Northern Regional Research Laboratory, Peoria, Illinois. One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.
[3] Bear Hybrid Corn Company, Inc., Decatur, Illinois.

has been determined by potentiometric titration with iodine (5); the highest amylose content yet found was 62%.

One strain of the series with the new endosperm type was grown in sufficient quantity for analysis and laboratory processing. The endosperm starch of the corn was 56% amylose. (This value is based on the assumption that corn amylose sorbs 200 mg. of iodine per gm.) The corn was found to contain 60% starch (by a polarimetric method) and 14.3% protein (Kjeldahl nitrogen $\times$ 6.25) on the moisture-free basis. A 74.4% recovery of the starch was obtained by a standard laboratory wet-milling procedure; the recovery was about the same as is obtained upon processing common dent corn by the method. The starch contained 1% protein (Kjeldahl nitrogen $\times$ 6.25) on the moisture-free basis. The relatively high protein content of the starch may have resulted from processing difficulty attributable to the fact that the corn had been harvested at high moisture content.

High-amylose starches from the series of corn samples are composed of granules comparable in birefringence to those of ordinary cornstarch and somewhat smaller in size. However, the high-amylose starches from the two crop years of the series have differed markedly from ordinary cornstarch in that the former contain many granules of irregular shape (Fig. 1). It is not yet known whether the irregularly

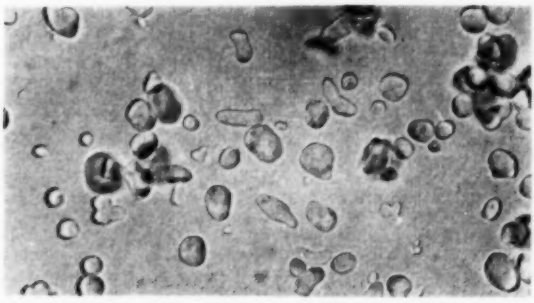

Fig. 1. Starch containing 56% amylose, from corn with high starch content. (350×)

shaped granules are characteristic of high-amylose starch in corn with high starch content or whether the granule shape is governed by genes that are independent of those governing the amylose content of the endosperm starch but which happen, in the cases found so far, to be associated with them.

Similarly to the high-amylose pea starches (5) and other high-amylose cornstarches previously reported (4), the high-amylose corn-

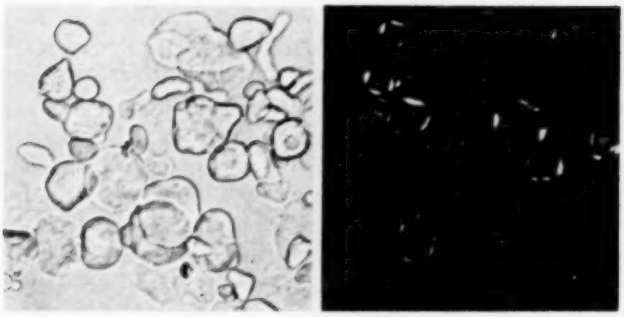

Fig. 2. Cornstarch containing 56% amylose. Water suspension heated in a boiling-water bath. The field between crossed nicols, at right, is the same as that at left. (380×)

starches reported here are only partially gelatinized when a water suspension of the granules is boiled (Fig. 2).

Further studies of the characteristics of these cornstarches are in progress, and breeding experiments are continuing with the aim of increasing still further the amylose content of corn endosperm starch.

Acknowledgments

The accompanying photomicrographs were taken by Mr. R. A. Larkin. Mrs. Margaret Holzapfel processed the corn sample for starch and made the analyses for starch and nitrogen.

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BOOK REVIEWS

Fertilizer Technology and Resources in the United States. Volume III of **Agronomy,** ed. by K. D. Jacob. 454 pp. Academic Press, Inc., New York (1953). Price $8.50.

This monograph gives the substance of a number of papers presented at a Short Course in Fertilizer Technology at the University of Maryland, August 21-25, 1950. Each contributor is an authority in his particular field and the volume has been ably edited by K. D. Jacob. Emphasis is placed on technological problems involved in the production of nitrogen, phosphate, and potash fertilizers both historically and those presently facing the industry. The various steps in each procedure are well described together with the chemical reactions which are involved. Numerous references are made to patented processes with an extensive bibliography at the end of each chapter.

The changing aspects of fertilizer technology are discussed in the first chapter, followed by three chapters on nitrogen production, needs, and supply. Three chapters are devoted to phosphate resources, chemistry, and the newer developments in processing. Potash resources and production are well described in two chapters. There is one chapter on the available sources and need of the secondary elements, calcium, magnesium, and sulfur. The next three chapters outline the general mechanics of mixed fertilizer production, physical and chemical problems in producing mixed fertilizers, and the production of mixed goods of high analysis. The concluding chapter discusses special fertilizers and their uses.

This monograph gives the substance of a number of papers presented at a in the fertilizer production field for the whys and wherefores involved in fertilizer production, and to anyone interested in the fundamentals of soil fertility and its maintenance.

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Hanna's Handbook of Agricultural Chemicals. By Lester W. Hanna. 209 pp. + vi. Lester W. Hanna, Rt. 1, Box 210, Forest Grove, Oregon, 1952. Price: $3.25.

This handbook contains descriptions of 96 fertilizers, 50 fumigants, 100 fungicides, 53 herbicides, 182 insecticides, 11 rodenticides and 13 miscellaneous materials. Chapters are included on official antidotes, toxicological information for several insecticides and tolerances for insecticide residues established by the Federal Food and Drug Administration, and a brief list of abbreviations of entomological terms.

The book gives information concerning the chemical nature, common names, physical and chemical properties, formulations and uses of the various agricultural chemicals which are listed, together with information on toxicity and the antidotes which are recommended in case of poisoning. A complete index of the various chemicals, classified according to their uses, is included.

Hanna's handbook provides a convenient source of information for those who are directly or indirectly concerned with agricultural chemicals.

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ERRATA

Cereal Chemistry, Vol. 30, No. 5

(September, 1953)

Page 358, Doyle C. Udy. Please insert the following line immediately after equation (2), line 10.

. . . where S_0 is the initial stress at the time, $t = 0$, and τ is the relaxation . . .

Page 361 Udy. Paragraph 3, last line: *for* t in the equation, *read* τ.

Page 385, Cole and Milner. Line 3, *for* 225 $m\mu$ *read* 245 $m\mu$.

The authors have courteously absolved the editorial staff and the printer of responsibility for this error, which occurred in a retyping of the manuscript.

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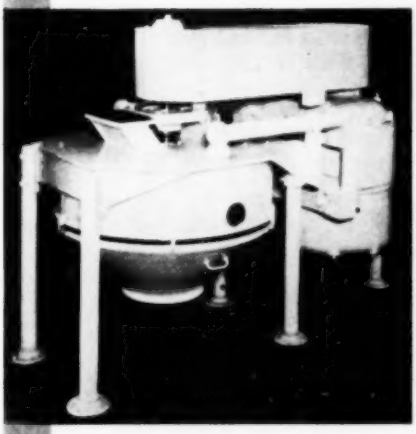

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